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(54) Title: CYTOTOXIC T-LYMPHOCYTE-INDUCING IMMUNOGENS FOR PREVENTION, TREATMENT, AND DIAGNOSIS OF CANCER

(57) Abstract: The present invention relates to compositions and methods for the prevention, treatment, and diagnosis of cancer, especially carcinomas, such as ovarian carcinoma. The invention discloses peptides, polypeptides, and polynucleotides that can be used to stimulate a CTL response against cancer.

WO 2007/150077 A2

CYTOTOXIC T-LYMPHOCYTE-INDUCING IMMUNOGENS FOR PREVENTION, TREATMENT, AND DIAGNOSIS OF CANCER

Field of the Invention

The present invention relates generally to the field of immunogens whose structures incorporate polypeptides comprising epitopic peptides derived from proteins expressed by cancer cells and to uses of said immunogens in eliciting cytotoxic T lymphocyte (CTL) responses for the diagnosis, prevention and treatment of cancer, preferably carcinoma, most preferably ovarian carcinoma.

Background of the Invention

The mammalian immune system has evolved a variety of mechanisms to protect the host from cancerous cells, an important component of this response being mediated by cells referred to as T cells. Cytotoxic T lymphocytes (CTLs) are specialized T cells that function primarily by recognizing and killing cancerous cells or infected cells, but also by secreting soluble molecules referred to as cytokines that can mediate a variety of effects on the immune system.

Evidence suggests that immunotherapy designed to stimulate a tumor-specific CTL response would be effective in controlling cancer. For example, it has been shown that human CTLs recognize sarcomas (Slovin, S. F. et al., J.Immunol., 137:3042-3048, (1987)), renal cell carcinomas (Schendel, D. J. et al., J.Immunol., 151:4209-4220, (1993)), colorectal carcinomas (Jacob, L. et al., Int.J.Cancer, 71:325-332, (1997)), ovarian carcinomas

(Loannides, C. G. et al., J.Immunol., 146:1700-1707, (1991)) (Peoples, G. E. et al., Surgery, 114:227-234, (1993)), pancreatic carcinomas (Peiper, M. et al., Eur.J.Immunol., 27:1115-1123, (1997); Wolfel, T. et al., Int.J.Cancer, 54:636-644, (1993)), squamous tumors of the head and neck (Yasumura, S. et al., Cancer Res., 53:1461-1468, (1993)), and squamous carcinomas of the lung (Slingluff, C. L. Jr et al., Cancer Res., 54:2731-2737, (1994); Yoshino, I. et al., Cancer Res., 54:3387-3390, (1994)). The largest number of reports of human tumor-reactive CTLs have concerned cancers (Boon, T. et al., Ann.Rev.Immunol., 12:337-365, (1994)). The ability of tumor-specific CTLs to mediate tumor regression, in both human (Rosenberg, S. A. et al., N.Engl.J.Med., 319:1676-1680, (1988)) and animal models (Celluzzi, C. M. et al., J.Exp.Med., 183:283-287, (1996); Mayordomo, J. I. et al., Nat.Med., 1:1297-1302, (1995); Zitvogel, L. et al., J.Exp.Med., 183:87-97, (1996)), suggests that methods directed at increasing CTL activity would likely have a beneficial effect with respect to tumor treatment.

In order for CTLs to kill or secrete cytokines in response to a cancer cell, the CTL must first recognize that cell as being cancerous. This process involves the interaction of the T cell receptor, located on the surface of the CTL, with what is generically referred to as an MHC-peptide complex which is located on the surface of the cancerous cell. MHC (major histocompatibility-complex)-encoded molecules have been subdivided into two types, and are referred to as class I and class II MHC-encoded molecules.

In the human immune system, MHC molecules are referred to as human leukocyte antigens (HLA). Within the MHC, located on chromosome six, are three different genetic loci that encode for class I MHC molecules. MHC molecules encoded at these loci are referred to as HLA-A, HLA-B, and HLA-C. The genes that can be encoded at each of these loci are extremely polymorphic, and thus, different individuals within the population express different class I MHC molecules on the surface of their cells. HLA-A1, HLA-A2, HLA-A3, HLA-B7, and HLA-B8 are examples of different class I MHC molecules that can be expressed from these loci. The present disclosure involves peptides that are associated with the HLA-A1, HLA-A2, or HLA-A11 molecules, HLA-A1 supertypes, HLA-A2 supertypes, and HLA-A11 supertypes and with the gene and protein that gives rise to these peptides. A supertype is a group of HLA molecules that present at least one shared epitope.

The peptides that associate with the MHC molecules can either be derived from proteins made within the cell, in which case they typically associate with class I MHC molecules (Rock, K. L. and Golde, U., Ann.Rev.Immunol., 17:739-779, (1999)) or they can be derived from proteins that are acquired from outside of the cell, in which case they

typically associate with class II MHC molecules (Watts, C., Ann.Rev.Immunol., 15:821-850, (1997)). Peptides that evoke a cancer-specific CTL response most typically associate with class I MHC molecules. The peptides that associate with a class I MHC molecule are typically nine amino acids in length, but can vary from a minimum length of eight amino acids to a maximum of fourteen amino acids in length. A class I MHC molecule with its bound peptide, or a class II MHC molecule with its bound peptide, is referred to as an MHC-peptide complex.

The process by which intact proteins are degraded into peptides is referred to as antigen processing. Two major pathways of antigen processing occur within cells (Rock, K. L. and Golde, U., Ann.Rev.Immunol., 17:739-779, (1999); Watts, C., Ann.Rev.Immunol., 15:821-850, (1997)). One pathway, which is largely restricted to cells that are antigen presenting cells such as dendritic cells, macrophages, and B cells, degrades proteins that are typically phagocytosed or endocytosed into the cell. Peptides derived in this pathway typically bind to class II MHC molecules. A second pathway of antigen processing is present in essentially all cells of the body. This second pathway primarily degrades proteins that are made within the cells, and the peptides derived from this pathway primarily bind to class I MHC molecules. It is the peptides from this second pathway of antigen processing that are referred to herein. Antigen processing by this latter pathway involves polypeptide synthesis and proteolysis in the cytoplasm. The peptides produced are then transported into the endoplasmic reticulum of the cell, associate with newly synthesized class I MHC molecules, and the resulting MHC-peptide complexes are then transported to the cell surface. Peptides derived from membrane and secreted proteins have also been identified. In some cases these peptides correspond to the signal sequence of the proteins that are cleaved from the protein by the signal peptidase. In other cases, it is thought that some fraction of the membrane and secreted proteins are transported from the endoplasmic reticulum into the cytoplasm where processing subsequently occurs.

Once bound to the class I MHC molecule and displayed on the surface of a cell, the peptides are recognized by antigen-specific receptors on CTLs. Mere expression of the class I MHC molecule itself is insufficient to trigger the CTL to kill the target cell if the antigenic peptide is not bound to the class I MHC molecule. Several methods have been developed to identify the peptides recognized by CTL, each method relying on the ability of a CTL to recognize and kill only those cells expressing the appropriate class I MHC molecule with the peptide bound to it (Rosenberg, S. A., Immunity, 10:281-287, (1999)). Such peptides can be derived from a non-self source, such as a pathogen (for example, following the

infection of a cell by a bacterium or a virus) or from a self-derived protein within a cell, such as a cancerous cell. Examples of sources of self-derived proteins in cancerous cells have been reviewed (Gilboa, E., Immunity, 11:263-270, (1999); Rosenberg, S. A., Immunity, 10:281-287, (1999)) and include: (i) mutated genes; (ii) aberrantly expressed genes such as an alternative open reading frame or through an intron-exon boundary; (iii) normal genes that are selectively expressed in only the tumor and the testis; and (iv) normal differentiation genes that are expressed in the tumor and the normal cellular counterpart.

Four different methodologies have typically been used for identifying the peptides that are recognized by CTLs. These are: (i) the genetic method; (2) motif analysis; (3) SErological analysis of REcombinant cDNA expression libraries (SEREX™); and (iv) the analytical chemistry approach or the Direct Identification of Relevant Epitopes for Clinical Therapeutics (DIRECT™).

The genetic method is an approach in which progressively smaller subsets of cDNA libraries from tumor cells are transfected into cells that express the appropriate MHC molecule but not the tumor-specific epitope. The molecular clones encoding T cell epitopes are identified by their ability to reconstitute tumor specific T cell recognition of transfected cells. The exact T cell epitope is then identified by a combination of molecular subcloning and the use of synthetic peptides based on the predicted amino acid sequence. Such methods, however, are susceptible to inadvertent identification of cross-reacting peptides, and are not capable of identifying important post-translational modifications.

Motif analysis involves scanning a protein for peptides containing known class I MHC binding motifs, followed by synthesis and assay of the predicted peptides for their ability to be recognized by tumor-specific CTL. This approach requires prior knowledge of the protein from which the peptides are derived. This approach is also greatly hampered by the fact that not all of the predicted peptide epitopes are presented on the surface of a cell (Yewdell, J. W. and Bennink, J. R., Ann.Rev.Immunol., 17:51-88, (1999)), thus additional experimentation is required to determine which of the predicted epitopes is useful.

The SEREX™ approach relies on using antibodies in the serum of cancer patients to screen cDNA expression libraries for a clone that expresses a protein recognized by the antibody. This methodology presumes that an antibody response will necessarily have developed in the presence of a T cell response, and thus, the identified clone is good candidate to encode a protein that can be recognized by T cells.

DIRECT™ involves a combination of cellular immunology and mass spectrometry. This approach involves the actual identification of CTL epitopes by sequencing the

naturally occurring peptides associated with class I MHC molecules. In this approach, cells are first lysed in a detergent solution, the peptides associated with the class I MHC molecules are purified, and the peptides fractionated by high performance liquid chromatography (HPLC). The peptides are then used to reconstitute recognition by tumor-specific CTLs on a non-tumor cell expressing the appropriate MHC molecules. Sequencing is readily performed by tandem mass spectrometry (Henderson, R. A. et al., Proc.Natl.Acad.Sci.U.S.A. 90:10275-10279, (1993); Hogan, K. T. et al., Cancer Res., 58:5144-5150, (1998); Hunt, D. F. et al., Science, 255:1261-1263, (1992); Slingluff, C. L. Jr et al., J.Immunol., 150:2955-2963, (1993)).

Immunization with cancer-derived, class I MHC-encoded molecule-associated peptides, or with a precursor polypeptide or protein that contains the peptide, or with a gene that encodes a polypeptide or protein containing the peptide, are forms of immunotherapy that can be employed in the treatment of cancer. These forms of immunotherapy require that immunogens be identified so that they can be formulated into an appropriate vaccine. Although a variety of cancer-derived antigens have been identified (Rosenberg, S. A., Immunity, 10:281-287, (1999)), not all of these are appropriate for broad-based immunotherapy as the expression of some peptides is limited to the tumor derived from a specific patient. Furthermore, the number of class I MHC molecules from which tumor-derived peptides have been discovered is largely restricted to HLA-A2. Thus, it would be useful to identify additional peptides that complex with class I MHC molecules other than HLA-A2. Such peptides would be particularly useful in the treatment of cancer patients who do not express the HLA-A2 molecule, HLA-A1 or HLA-A11 antigens, HLA-A1 supertypes, HLA-A2 supertypes and HLA-A11 supertypes, for example. It is also particularly useful to identify antigenic peptides that are derived from different original proteins, even if the derived peptides associate with the same class I MHC molecule. Because an active immune response can result in the outgrowth of tumor cells that have lost the expression of a particular precursor protein for a given antigenic peptide, it is advantageous to stimulate an immune response against peptides derived from more than one protein, as the chances of the tumor cell losing the expression of both proteins is the multiple of the chances of losing each of the individual proteins.

Summary of the Invention

The present invention relates to Immunogens comprising polypeptides with amino acid sequences comprising epitopic sequences selected from the sequences of SEQ ID NO:

1 to 354 and which immunogens facilitate a cytotoxic T lymphocyte (CTL)-mediated immune response against cancers. The present invention also relates to nucleic acid molecules that encode for the polypeptides and/or the full length proteins from which the polypeptides are derived, of such immunogens, and which can also be used to facilitate an immune response against cancer.

The present invention provides compositions comprising the immunogen described herein, and polynucleotides that direct the synthesis of such polypeptides, whereby the oligopeptides and polypeptides of such immunogens are capable of inducing a CTL response against cells expressing a protein comprising an epitopic sequence of at least one of SEQ ID NO: 1 to 354. The cells are usually cancer cells, preferably carcinoma cells, most preferably ovarian carcinomas expressing such proteins.

The present invention further relates to polynucleotides comprising the gene coding for a polypeptide of the immunogens disclosed herein.

The present invention also provides methods that comprise contacting a lymphocyte, especially a CTL, with an immunogen of the invention under conditions that induce a CTL response against a tumor cell, and more specifically against a cancer cell. The methods may involve contacting the CTL with the immunogenic peptide in vivo, in which case the peptides, polypeptides, and polynucleotides of the invention are used as vaccines, and will be delivered as a pharmaceutical composition comprising a pharmaceutically acceptable carrier and the immunogen, typically along with an adjuvant or one or more cytokines.

Alternatively, the immunogens of the present invention can be used to induce a CTL response in vitro. The generated CTL can then be introduced into a patient with cancer, more specifically cancer, colorectal carcinoma, ovarian carcinoma, breast carcinoma, lung carcinoma, or prostate carcinoma. Alternatively, the ability to generate CTL in vitro could serve as a diagnostic for cancer generally, including colorectal carcinoma, ovarian carcinoma, breast carcinoma, lung carcinoma, or prostate carcinoma.

Detailed Description of the Invention

Definitions

As used herein and except as noted otherwise, all terms are defined as given below.

The term "peptide" is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The peptides are typically 9 amino acids in

length, but can be as short as 8 amino acids in length, and as long as 14 amino acids in length.

The term "oligopeptide" is used herein to designate a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The length of the oligopeptide is not critical to the invention as long as the correct epitope or epitopes are maintained therein. The oligopeptides are typically less than about 30 amino acid residues in length, and greater than about 14 amino acids in length.

The term "polypeptide" designates a series of amino acid residues, connected one to the other typically by peptide bonds between the alpha-amino and carbonyl groups of the adjacent amino acids. The length of the polypeptide is not critical to the invention as long as the correct epitopes are maintained. In contrast to the terms peptide or oligopeptide, the term polypeptide is meant to refer to protein molecules of longer than about 30 residues in length.

A peptide, oligopeptide, protein, or polynucleotide coding for such a molecule is "immunogenic" (and thus an "immunogen" within the present invention) if it is capable of inducing an immune response. In the case of the present invention, immunogenicity is more specifically defined as the ability to induce a CTL-mediated response. Thus, an "immunogen" would be a molecule that is capable of inducing an immune response, and in the case of the present invention, a molecule capable of inducing a CTL response.

A T cell "epitope" is a short peptide molecule that binds to a class I or II MHC molecule and that is subsequently recognized by a T cell. T cell epitopes that bind to class I MHC molecules are typically 8-14 amino acids in length, and most typically 9 amino acids in length. T cell epitopes that bind to class II MHC molecules are typically 12-20 amino acids in length. In the case of epitopes that bind to class II MHC molecules, the same T cell epitope may share a common core segment, but differ in the length of the carboxy- and amino-terminal flanking sequences due to the fact that ends of the peptide molecule are not buried in the structure of the class II MHC molecule peptide-binding cleft as they are in the class I MHC molecule peptide-binding cleft.

There are three different genetic loci that encode for class I MHC molecules: HLA-A, HLA-B, and HLA-C. HLA-A1, HLA-A2, and HLA-A11 are examples of different class I MHC molecules that can be expressed from these loci. The present invention also involves peptides that are associated with HLA-A1 supertypes, HLA-A2 supertypes, and HLA-A11 supertypes. A supertype is a group of HLA molecules that present at least one

shared epitope. MHC molecule peptides that have been found to bind to one member of the MHC allele supertype family (A1 for example) are thought to be likely to bind to other members of the same supertype family (A32 for example; see Talbe 1, below.

Table 1.

Supertype	Motif	Genotypes
A1	x[TI(SVLM)] xxxxxx[WFY]	A*0101, A*0102, A*2501, A*2601, A*2604, A*3201, A*3601, A*4301, A*8001
A2	x[LIVMATQ] xxxxxx[LIVMAT]	A*0201, A*0202, A*0203, A*0204, A*0205, A*0206, A*0207, A*6802, A*6901
A3	x[AILMVGT] xxxxxx[RK]	A*0301, A*1101, A*3101, A*3301, A*6801
A24	x[YF(WIVLMT)] xxxxxx[FI(YWLM)]	A*2301, A*2402, A*2403, A*2404, A*3001, A*3002, A*3003
B7	x[P]xxxxxx [ALIMVFWY]	B*0702, B*0703, B*0704, B*0705, B*1508, B*3501, B*3502, B*3503, B*51, B*5301, B*5401, B*5501, B*5502, B*5601, B*5602, B*6701, B*7801
B27	x[RKH]xxxxxx [FLY(WMI)]	B*1401, B*1402, B*1503, B*1509, B*1510, B*1518, B*2701, B*2702, B*2703, B*2704, B*2705, B*2706, B*2707, B*2708, B*3801, B*3802, B*3901, B*3902, B*3903, B*3904, B*4801, B*4802, B*7301
B44	x[E(D)]xxxxxx [FWYLIMVA]	B*18, B*3701, B*4001, B*4006, B*4101, B*4402, B*4403, B*4501, B*4901, B*5001
B58	x[AST]xxxxxx [FWY(LIV)]	B*1516, B*1517, B*5701, B*5702, B*58
B62	x[QL(IVMP)] xxxxxx[FWY(MIV)]	B*1301, B*1302, B*1501, B*1502, B*1506, B*1512, B*1513, B*1514, B*1519, B*1521, B*4601, B*52

As used herein, reference to a DNA sequence includes both single stranded and double stranded DNA. Thus, the specific sequence, unless the context indicates otherwise, refers to the single strand DNA of such sequence, the duplex of such sequence with its complement (double stranded DNA) and the complement of such sequence.

The term "coding region" refers to that portion of a gene which either naturally or normally codes for the expression product of that gene in its natural genomic environment, i.e., the region coding in vivo for the native expression product of the gene. The coding region can be from a normal, mutated or altered gene, or can even be from a DNA sequence, or gene, wholly synthesized in the laboratory using methods well known to those of skill in the art of DNA synthesis.

The term "nucleotide sequence" refers to a heteropolymer of deoxyribonucleotides. The nucleotide sequence encoding for a particular peptide, oligopeptide, or polypeptide may

be naturally occurring or they may be synthetically constructed. Generally, DNA segments encoding the peptides, polypeptides, and proteins of this invention are assembled from cDNA fragments and short oligonucleotide linkers, or from a series of oligonucleotides, to provide a synthetic gene which is capable of being expressed in a recombinant transcriptional unit comprising regulatory elements derived from a microbial or viral operon.

The term "expression product" means that polypeptide or protein that is the natural translation product of the gene and any nucleic acid sequence coding equivalents resulting from genetic code degeneracy and thus coding for the same amino acid(s).

The term "fragment," when referring to a coding sequence, means a portion of DNA comprising less than the complete coding region whose expression product retains essentially the same biological function or activity as the expression product of the complete coding region.

The term "DNA segment" refers to a DNA polymer, in the form of a separate fragment or as a component of a larger DNA construct, which has been derived from DNA isolated at least once in substantially pure form, i.e., free of contaminating endogenous materials and in a quantity or concentration enabling identification, manipulation, and recovery of the segment and its component nucleotide sequences by standard biochemical methods, for example, by using a cloning vector. Such segments are provided in the form of an open reading frame uninterrupted by internal nontranslated sequences, or introns, which are typically present in eukaryotic genes. Sequences of non-translated DNA may be present downstream from the open reading frame, where the same do not interfere with manipulation or expression of the coding regions.

The term "primer" means a short nucleic acid sequence that is paired with one strand of DNA and provides a free 3'OH end at which a DNA polymerase starts synthesis of a deoxyribonucleotide chain.

The term "promoter" means a region of DNA involved in binding of RNA polymerase to initiate transcription.

The term "open reading frame (ORF)" means a series of triplets coding for amino acids without any termination codons and is a sequence (potentially) translatable into protein.

The term "isolated" means that the material is removed from its original environment (e.g., the natural environment if it is naturally occurring). For example, a naturally-occurring polynucleotide or polypeptide present in a living animal is not isolated,

but the same polynucleotide or polypeptide, separated from some or all of the coexisting materials in the natural system, is isolated. Such polynucleotides could be part of a vector and/or such polynucleotides or polypeptides could be part of a composition, and still be isolated in that such vector or composition is not part of its natural environment.

The polynucleotides, and recombinant or immunogenic polypeptides, disclosed in accordance with the present invention may also be in "purified" form. The term "purified" does not require absolute purity; rather, it is intended as a relative definition, and can include preparations that are highly purified or preparations that are only partially purified, as those terms are understood by those of skill in the relevant art. For example, individual clones isolated from a cDNA library have been conventionally purified to electrophoretic homogeneity. Purification of starting material or natural material to at least one order of magnitude, preferably two or three orders, and more preferably four or five orders of magnitude is expressly contemplated. Furthermore, the claimed polypeptide which has a purity of preferably 0.001%, or at least 0.01% or 0.1%; and even desirably 1% by weight or greater is expressly contemplated.

The nucleic acids and polypeptide expression products disclosed according to the present invention, as well as expression vectors containing such nucleic acids and/or such polypeptides, may be in "enriched form." As used herein, the term "enriched" means that the concentration of the material is at least about 2, 5, 10, 100, or 1000 times its natural concentration (for example), advantageously 0.01%, by weight, preferably at least about 0.1% by weight. Enriched preparations of about 0.5%, 1%, 5%, 10%, and 20% by weight are also contemplated. The sequences, constructs, vectors, clones, and other materials comprising the present invention can advantageously be in enriched or isolated form.

The term "active fragment" means a fragment that generates an immune response (i.e., has immunogenic activity) when administered, alone or optionally with a suitable adjuvant, to an animal, such as a mammal, for example, a rabbit or a mouse, and also including a human, such immune response taking the form of stimulating a CTL response within the recipient animal, such as a human. Alternatively, the "active fragment" may also be used to induce a CTL response in vitro.

As used herein, the terms "portion," "segment," and "fragment," when used in relation to polypeptides, refer to a continuous sequence of residues, such as amino acid residues, which sequence forms a subset of a larger sequence. For example, if a polypeptide were subjected to treatment with any of the common endopeptidases, such as trypsin or chymotrypsin, the oligopeptides resulting from such treatment would represent portions,

segments or fragments of the starting polypeptide. This means that any such fragment will necessarily contain as part of its amino acid sequence a segment, fragment or portion, that is substantially identical, if not exactly identical, to a sequence of SEQ ID NO: 355 to 693, which correspond to the naturally occurring, or "parent" proteins of the SEQ ID NO: 1 to 354. When used in relation to polynucleotides, such terms refer to the products produced by treatment of said polynucleotides with any of the common endonucleases.

In accordance with the present invention, the term "percent identity" or "percent identical," when referring to a sequence, means that a sequence is compared to a claimed or described sequence after alignment of the sequence to be compared (the "Compared Sequence") with the described or claimed sequence (the "Reference Sequence"). The Percent Identity is then determined according to the following formula:

Percent Identity=100 [1-(C/R)]

wherein C is the number of differences between the Reference Sequence and the Compared Sequence over the length of alignment between the Reference Sequence and the Compared Sequence wherein (i) each base or amino acid in the Reference Sequence that does not have a corresponding aligned base or amino acid in the Compared Sequence and (ii) each gap in the Reference Sequence and (iii) each aligned base or amino acid in the Reference Sequence that is different from an aligned base or amino acid in the Compared Sequence, constitutes a difference; and R is the number of bases or amino acids in the Reference Sequence over the length of the alignment with the Compared Sequence with any gap created in the Reference Sequence also being counted as a base or amino acid.

If an alignment exists between the Compared Sequence and the Reference Sequence for which the percent identity as calculated above is about equal to or greater than a specified minimum Percent Identity then the Compared Sequence has the specified minimum percent identity to the Reference Sequence even though alignments may exist in which the herein above calculated Percent Identity is less than the specified Percent Identity.

The present invention relates generally to immunogens and immunogenic compositions, and methods of use therefore, for the prevention, treatment, and diagnosis of cancer, especially carcinomas, including ovarian carcinomas. Disclosed according to the invention are immunogens comprising proteins or polypeptides whose amino acid sequences comprises one or more epitopic oligopeptides with sequences selected from the group SEQ ID NO: 1 to 354. In addition, the invention further relates to polynucleotides

that can be used to stimulate a CTL response against cancer, and more specifically carcinoma, especially ovarian carcinomas.

In accordance with the present invention there are disclosed specific oligopeptide sequences with amino acid sequences shown in SEQ ID NO: 1 to 354, which represent epitopic peptides (i.e. immunogenic oligopeptide sequences) of at least about 8 amino acids in length, preferably about 9 amino acids in length (i.e., nonapeptides), and no longer than about 10 amino acids in length and present as part of a larger structure, such as a polypeptide or full length protein.

The polypeptides forming the immunogens of the present invention have amino acid sequences that comprise at least one stretch, possibly two, three, four, or more stretches of about 8 to 10 residues in length and which stretches differ in amino acid sequence from the sequences of SEQ ID NO: 1 to 354 by no more than about 1 amino acid residue, preferably a conservative amino acid residue, especially amino acids of the same general chemical character, such as where they are hydrophobic amino acids.

Said polypeptides can be of any desired length so long as they have immunogenic activity in that they are able, under a given set of desirable conditions, to elicit in vitro or in vivo the activation of cytotoxic T lymphocytes (CTLs) (i.e., a CTL response) against a presentation of a cancer specific protein, especially a carcinoma or sarcoma specific protein, most especially MAGE D, MAGE 4, MFG-E8 or human retinoblastoma-like protein, especially when such proteins are presented along with MHC-I proteins, such as where said proteins are presented in vitro or in vivo by an antigen presenting cell (APC). The proteins and polypeptides forming the immunogens of the present invention can be naturally occurring or may be synthesized chemically. According to the present invention the polypeptides may comprise at least one of SEQ ID NO: 355 to 693.

The present invention is also directed to an isolated polypeptide, especially one having immunogenic activity, the sequence of which comprises within it one or more stretches comprising any 2 or more of the sequences of SEQ ID NO: 1 to 354 and in any relative quantities and wherein said sequences may differ by one amino acid residues from the sequences of SEQ ID NO: 1 to 354 in any given stretch of 8 to 10 amino acid residues. Thus, within the present invention, by way of a non-limiting example only, such polypeptide may contain as part of its amino acid sequence, nonapeptide fragments having up to 8 amino acids identical to a sequence of SEQ ID NO: 1-4 such that the polypeptide comprises, in a specific embodiment, 2 segments with at least 8 residues identical to SEQ ID NO: 1 and one segment with at least 8 residues identical to SEQ ID NO: 3. In other

embodiments, other combinations and permutations of the epitopic sequences disclosed herein may be part of an immunogen of the present invention or of such a polypeptide so long as any such polypeptide comprises at least 2 such epitopes, whether such epitopes are different or the same. Thus, in a specific embodiment, a polypeptide of the present invention may comprise 2 copies of the sequence of SEQ ID NO: 2 at some point or points within its length. Of course, any combinations and permutations of the epitopes disclosed herein, as long as they are present at least two in number in such polypeptides, are expressly contemplated.

All of the epitopic peptides of SEQ ID NO: 1 to 354 are derived from proteins expressed by cancer cells and sequences and were identified through the method of Automated High Through-put Sequencing (HTPS). Accordingly, SEQ ID NO: 355 to 693 are polypeptides that comprise at least one of SEQ ID NO: 1 to 354.

Oligopeptides as disclosed herein may themselves be prepared by methods well known to those skilled in the art. (Grant, G. A., Synthetic Peptides: A User's Guide, 1992, W. H. Freeman and Company, New York; Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York).

Besides the sequences of SEQ ID NO:1 to 354, the proteins and polypeptides forming the immunogens of the present invention may also comprise one or more other immunogenic amino acid stretches known to be associated with cancer, and more specifically with carcinomas and melanomas, including colorectal carcinoma, ovarian carcinoma, breast carcinoma, lung carcinoma, or prostate carcinoma, and which may stimulate a CTL response whereby the immunogenic peptides associate with HLA-A1 or HLA-A11, or HLA-A2, or another class I MHC (i.e., MHC-1) molecule.

The immunogens of the present invention can be in the form of a composition of one or more of the different immunogens and wherein each immunogen is present in any desired relative abundance. Such compositions can be homogeneous or heterogeneous with respect to the individual immunogenic peptide components present therein, having only one or more than one of such peptides.

The oligopeptides and polypeptides useful in practicing the present invention may be derived by fractionation of naturally occurring proteins by methods such as protease treatment, or they may be produced by recombinant or synthetic methodologies that are well known and clear to the skilled artisan (Ausubel, F. M. et al, Current Protocols in Molecular Biology, 1999, John Wiley & Sons, Inc., New York; Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York; Molecular Cloning: A

Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor). The polypeptide may comprise a recombinant or synthetic polypeptide that comprises at least one of SEQ ID NO: 1 to 354 which sequences may also be present in multiple copies. Thus, oligopeptides and polypeptides of the present invention may have one, two, three, or more such immunogenic peptides within the amino acid sequence of said oligopeptides and polypeptides, and said immunogenic peptides, or epitopes, may be the same or may be different, or may have any number of such sequences wherein some of them are identical to each other in amino acid sequence while others within the same polypeptide sequence are different from each other and said epitopic sequences may occur in any order within said immunogenic polypeptide sequence. The location of such sequences within the sequence of a polypeptide forming an immunogen of the invention may affect relative immunogenic activity. In addition, immunogens of the present invention may comprise more than one protein comprising the amino acid sequences disclosed herein. Such polypeptides may be part of a single composition or may themselves be covalently or non-covalently linked to each other.

The immunogenic peptides disclosed herein may also be linked directly to, or through a spacer or linker to: an immunogenic carrier such as serum albumin, tetanus toxoid, keyhole limpet hemocyanin, dextran, or a recombinant virus particle; an immunogenic peptide known to stimulate a T helper cell type immune response; a cytokine such as interferon gamma or GMCSF; a targeting agent such as an antibody or receptor ligand; a stabilizing agent such as a lipid; or a conjugate of a plurality of epitopes to a branched lysine core structure, such as the so-called "multiple antigenic peptide" described in (Posneft, D. N. et al., J.Biol.Chem., 263;1719-1725, (1988)); a compound such as polyethylene glycol to increase the half life of the peptide; or additional amino acids such as a leader or secretory sequence, or a sequence employed for the purification of the mature sequence. Spacers and linkers are typically comprised of relatively small, neutral molecules, such as amino acids and which are substantially uncharged under physiological conditions. Such spacers are typically selected from the group of nonpolar or neutral polar amino acids, such as glycine, alanine, serine and other similar amino acids. Such optional spacers or linkers need not be comprised of the same residues and thus may be either homo- or hetero-oligomers. When present, such linkers will commonly be of length at least one or two, commonly 3, 4, 5, 6, and possibly as much as 10 or even up to 20 residues (in the case of amino acids). In addition, such linkers need not be composed of amino acids but any oligomeric structures will do as well so long as they provide the correct spacing so as to

optimize the desired level of immunogenic activity of the immunogens of the present invention. The immunogen may therefore take any form that is capable of eliciting a CTL response.

In addition, the immunogenic peptides of the present invention may be part of an immunogenic structure via attachments other than conventional peptide bonds. Thus, any manner of attaching the peptides of the invention to an immunogen of the invention, such as an immunogenic polypeptide as disclosed herein, could provide an immunogenic structure as claimed herein. Thus, immunogens, such as proteins of the invention, are structures that contain the peptides disclosed according to the present invention but such immunogenic peptides may not necessarily be attached thereto by the conventional means of using ordinary peptide bounds. The immunogens of the present invention simply contain such peptides as part of their makeup, but how such peptides are to be combined to form the final immunogen is left to the talent and imagination of the user and is in no way restricted or limited by the disclosure contained herein.

The peptides that are naturally processed and bound to a class I MHC molecule, and which are recognized by a tumor-specific CTL, need not be the optimal peptides for stimulating a CTL response. See, for example, (Parkhurst, M. R. et al., J.Immunol., 157:2539-2548, (1996); Rosenberg, S. A. et al., Nat.Med., 4:321-327, (1998)). Thus, there can be utility in modifying a peptide, such that it more readily induces a CTL response. Generally, peptides may be modified at two types of positions. The peptides may be modified at amino acid residues that are predicted to interact with the class I MHC molecule, in which case the goal is to create a peptide that has a higher affinity for the class I MHC molecule than does the original peptide. The peptides can also be modified at amino acid residues that are predicted to interact with the T cell receptor on the CTL, in which case the goal is to create a peptide that has a higher affinity for the T cell receptor than does the original peptide. Both of these types of modifications can result in a variant peptide that is related to an original peptide, but which is better able to induce a CTL response than is the original peptide. As used herein, the term "original peptide" means an oligopeptide with the amino acid sequence selected from SEQ ID NO: 1 to 354.

The original peptides disclosed herein can be modified by the substitution of one or more residues at different, possibly selective, sites within the peptide chain. Such substitutions may be of a conservative nature, for example, where one amino acid is replaced by an amino acid of similar structure and characteristics, such as where a hydrophobic amino acid is replaced by another hydrophobic amino acid. Even more

conservative would be replacement of amino acids of the same or similar size and chemical nature, such as where leucine is replaced by isoleucine. In studies of sequence variations in families of naturally occurring homologous proteins, certain amino acid substitutions are more often tolerated than others, and these are often show correlation with similarities in size, charge, polarity, and hydrophobicity between the original amino acid and its replacement, and such is the basis for defining "conservative substitutions."

Conservative substitutions are herein defined as exchanges within one of the following five groups: Group 1--small aliphatic, nonpolar or slightly polar residues (Ala, Ser, Thr, Pro, Gly); Group 2--polar, negatively charged residues and their amides (Asp, Asn, Glu, Gln); Group 3--polar, positively charged residues (His, Arg, Lys); Group 4--large, aliphatic, nonpolar residues (Met, Leu, Ile, Val, Cys); and Group 4--large, aromatic residues (Phe, Tyr, Trp).

Less conservative substitutions might involve the replacement of one amino acid by another that has similar characteristics but is somewhat different in size, such as replacement of an alanine by an isoleucine residue. Highly nonconservative replacements might involve substituting an acidic amino acid for one that is polar, or even for one that is basic in character. Such radical substitutions cannot, however, be dismissed as potentially ineffective since chemical effects are not totally predictable and radical substitutions might well give rise to serendipitous effects not otherwise predictable from simple chemical principles.

Of course, such substitutions may involve structures other than the common L-amino acids. Thus, D-amino acids might be substituted for the L-amino acids commonly found in the antigenic peptides of the invention and yet still be encompassed by the disclosure herein. In addition, amino acids possessing non-standard R groups (i.e., R groups other than those found in the common 20 amino acids of natural proteins) may also be used for substitution purposes to produce immunogens and immunogenic polypeptides according to the present invention.

If substitutions at more than one position are found to result in a peptide with substantially equivalent or greater antigenic activity as defined below, then combinations of those substitutions will be tested to determine if the combined substitutions result in additive or syngeneic effects on the antigenicity of the peptide. At most, no more than 4 positions within the peptide would simultaneously be substituted.

Based on cytotoxicity assays, an epitope is considered substantially identical to the reference peptide if it has at least 10% of the antigenic activity of the reference peptide as

defined by the ability of the substituted peptide to reconstitute the epitope recognized by a CTL in comparison to the reference peptide. Thus, when comparing the lytic activity in the linear portion of the effector:target curves with equimolar concentrations of the reference and substituted peptides, the observed percent specific killing of the target cells incubated with the substituted peptide should be equal to that of the reference peptide at an effector:target ratio that is no greater than 10-fold above the reference peptide effector:target ratio at which the comparison is being made.

Preferably, when the CTLs specific for a peptide of SEQ ID NO:1 to 354 are tested against the substituted peptides, the peptide concentration at which the substituted peptides achieve half the maximal increase in lysis relative to background is no more than about 1 mM, preferably no more than about 1 μM, more preferably no more than about 1 nM, and still more preferably no more than about 100 pM, and most preferably no more than about 10 pM. It is also preferred that the substituted peptide be recognized by CTLs from more than one individual, at least two, and more preferably three individuals.

Thus, the epitopes of the present invention may be identical to naturally occurring tumor-associated or tumor-specific epitopes or may include epitopes that differ by no more than 4 residues from the reference peptide, as long as they have substantially identical antigenic activity.

It should be appreciated that an immunogen may consist only of a peptide of SEQ ID NO:1 to 354, or comprise a peptide of SEQ ID NO:1 to 354, or comprise a plurality of peptides selected from SEQ ID NO:1 to 354, or comprise a polypeptide that itself comprises one or more of the epitopic peptides of SEQ ID NO: 1 to 354.

The immunogenic peptides and polypeptides of the invention can be prepared synthetically, by recombinant DNA technology, or they can be isolated from natural sources such as tumor cells expressing the original protein product.

The polypeptides and oligopeptides disclosed herein can be synthesized in solution or on a solid support in accordance with conventional techniques. Various automated peptide synthesizers are commercially available and can be used in accordance with known protocols. See, for example, (Grant, G. A., Synthetic Peptides: A User's Guide, 1992, W. H. Freeman and Company, New York; Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York). Fragments of polypeptides of the invention can also be synthesized as intermediates in the synthesis of a larger polypeptide.

Recombinant DNA technology may be employed wherein a nucleotide sequence which encodes an immunogenic peptide or polypeptide of interest is inserted into an expression vector, transformed or transfected into an appropriate host cell, and cultivated under conditions suitable for expression. These procedures are well known in the art to the skilled artisan, as described in (Coligan, J. E. et al, Current Protocols in Immunology, 1999, John Wiley & Sons, Inc., New York; Ausubel, F. M. et al, Current Protocols in Molecular Biology, 1999, John Wiley & Sons, Inc., New York; Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor). Thus, recombinantly produced peptides or polypeptides can be used as the immunogens of the invention.

The coding sequences for peptides of the length contemplated herein can be synthesized on commercially available automated DNA synthesizers using protocols that are well know in the art. See for example, (Grant, G. A., Synthetic Peptides: A User's Guide, 1992, W. H. Freeman and Company, New York; Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York). The coding sequences can also be modified such that a peptide or polypeptide will be produced that incorporates a desired amino acid substitution. The coding sequence can be provided with appropriate linkers, be ligated into suitable expression vectors that are commonly available in the art, and the resulting DNA or RNA molecule can be transformed or transfected into suitable hosts to produce the desired fusion protein. A number of such vectors and suitable host systems are available, and their selection is left to the skilled artisan. For expression of the fusion proteins, the coding sequence will be provided with operably linked start and stop codons, promoter and terminator regions, and a replication system to provide an expression vector for expression in the desired host cell. For example, promoter sequences compatible with bacterial hosts are provided in plasmids containing convenient restriction sites for insertion of the desired coding sequence. The resulting expression vectors are transformed into suitable bacterial hosts. Of course, yeast, insect, and mammalian host cells may also be used, employing suitable vectors and control sequences.

Host cells are genetically engineered (transduced or transformed or transfected) with the vectors of this invention which may be, for example, a cloning vector or an expression vector. The vector may be, for example, in the form of a plasmid, a viral particle, a phage, etc. The engineered host cells can be cultured in conventional nutrient media modified as appropriate for activating promoters, selecting transformants or amplifying the genes of the present invention. The culture conditions, such as temperature, pH and the like, are those

previously used with the host cell selected for expression, and will be apparent to the ordinarily skilled artisan.

More particularly, the present invention also includes recombinant constructs comprising one or more of the sequences as broadly described above. The constructs comprise a vector, such as a plasmid or viral vector, into which a sequence of the invention has been inserted, in a forward or reverse orientation. In a preferred aspect of this embodiment, the construct further comprises regulatory sequences, including, for example, a promoter, operably linked to the sequence. Large numbers of suitable vectors and promoters are known to those of skill in the art, and are commercially available.

In a further embodiment, the present invention relates to host cells containing the above-described constructs. The host cell can be a higher eukaryotic cell, such as a mammalian cell, or a lower eukaryotic cell, such as a yeast cell, or the host cell can be a prokaryotic cell, such as a bacterial cell. Introduction of the construct into the host cell can be effected by calcium phosphate transfection, DEAE-Dextran mediated transfection, or electroporation (Ausubel, F. M. et al, Current Protocols in Molecular Biology, 1999, John Wiley & Sons, Inc., New York; Molecular Cloning: A Laboratory Manual, 1989, Cold Spring Harbor Laboratory Press, Cold Spring Harbor). Such cells can routinely be utilized for assaying CTL activity by having said genetically engineered, or recombinant, host cells express the immunogenic peptides of the present invention.

Various mammalian cell culture systems can also be employed to express recombinant protein. Examples of mammalian expression systems include the COS-7 lines of monkey kidney fibroblasts, described by Gluzman, Cell, 23:175 (1981), and other cell lines capable of expressing a compatible vector, for example, the C127, 3T3, CHO, HeLa and BHK cell lines. Mammalian expression vectors will comprise an origin of replication, a suitable promoter and enhancer, and also any necessary ribosome binding sites, polyadenylation site, splice donor and acceptor sites, transcriptional termination sequences, and 5' flanking non-transcribed sequences. DNA sequences derived from the SV40 splice, and polyadenylation sites may be used to provide the required nontranscribed genetic elements.

The polypeptide can be recovered and purified from recombinant cell cultures by methods including ammonium sulfate or ethanol precipitation, acid extraction, anion or cation exchange chromatography, phosphocellulose chromatography, hydrophobic interaction chromatography, affinity chromatography, hydroxylapatite chromatography and lectin chromatography. Protein refolding steps can be used, as necessary, in completing

configuration of the mature protein. High performance liquid chromatography (HPLC) can be employed for final purification steps.

The immunogenic peptides of the present invention may be used to elicit CTLs ex vivo from either healthy individuals or from cancer patients with cancer, such as colorectal carcinoma, lung carcinoma, ovarian carcinoma, breast carcinoma, or prostate carcinoma. Such responses are induced by incubating in tissue culture the individual's CTL precursor lymphocytes together with a source of antigen presenting cells and the appropriate immunogenic peptide. Examples of suitable antigen presenting cells include dendritic cells, macrophages, and activated B cells. Typically, the peptide at concentrations between 10 and 40μg/ml, would be pre-incubated with the antigen presenting cells for periods ranging from 1 to 18 hrs. β_2-microglobulin (4 μg/ml) can be added during this time period to enhance binding. The antigen presenting cells may also be held at room temperature during the incubation period (Ljunggren, H.-G. et al., Nature, 346:476-480, (1990)) or pretreated with acid (Zeh, H. J., III et al., Hum.Immunol., 39:79-86, (1994)) to promote the generation of denatured class I MHC molecules which can then bind the peptide. The precursor CTLs (responders) are then added to the antigen presenting cells to which the immunogenic peptide has bound (stimulators) at responder to stimulator ratios of between 5:1 and 50:1, and most typically between 10:1 and 20:1. The co-cultivation of the cells is carried out at 37° C. in RPMI 1640, 10% fetal bovine serum, 2 mM L-glutamine, and IL-2 (5-20 Units/ml). Other cytokines, such as IL-1, IL-7, and IL-12 may also be added to the culture. Fresh IL-2-containing media is added to the cultures every 2-4 days, typically by removing one-half the old media and replenishing it with an equal volume of fresh media. After 7-10 days, and every 7-10 days thereafter, the CTL are re-stimulated with antigen presenting cells to which immunogenic peptide has been bound as described above. Fresh IL-2-containing media is added to the cells throughout their culture as described above. Three to four rounds of stimulation, and sometimes as many five to eight rounds of stimulation, are required to generate a CTL response that can then be measured in vitro. The above described protocol is illustrative only and should not be considered limiting. Many in vitro CTL stimulation protocols have been described and the choice of which one to use is well within the knowledge of the skilled artisan. The peptide-specific CTL can be further expanded to large numbers by treatment with anti-CD3 antibody. For example, see (Riddell, S. R. and Greenberg, P. D., J.Immunol.Methods, 128:189-201, (1990); Walter, E. A. et al., N.Engl.J.Med., 333:1038-1044, (1995)).

Antigen presenting cells that are to be used to stimulate a CTL response are typically incubated with peptide of an optimal length, most commonly a nonapeptide, that allows for direct binding of the peptide to the class I MHC molecule without additional processing. Larger oligopeptides and polypeptides are generally ineffective in binding to class I MHC molecules as they are not efficiently processed into an appropriately sized peptide in the extracellular milieu. There a variety of approaches that are known in the art, however, that allow oligopeptides and polypeptides to be exogenously acquired by a cell, which then allows for their subsequent processing and presentation by a class I MHC molecule. Representative, but non-limiting examples of such approaches include electroporation of the molecules into the cell (Harding, C. H. III. Eur.J.Immunol., 22:1865-1869, (1992)), encapsulation of the molecules in liposomes which are fused to the cells of interest (Reddy, R. et al., J.Immunol.Methods, 141:157-163, (1991)), or osmotic shock in which the molecules are taken up via pinocytosis (Moore, M. W. et al., Cell, 54:777-785, (1988)). Thus, oligopeptides and polypeptides that comprise one or more of the peptides of the invention can be provided to antigen presenting cells in such a fashion that they are delivered to the cytoplasm of the cell, and are subsequently processed to allow presentation of the peptides.

Antigen presenting cells suitable for stimulating an in vitro CTL response that is specific for one or more of the peptides of the invention can also be prepared by introducing polynucleotide vectors encoding the sequences into the cells. These polynucleotides can be designed such that they express only a single peptide of the invention, multiple peptides of the invention, or even a plurality of peptides of the invention. There are a variety of approaches that are known in the art, that allow polynucleotides to be introduced and expressed in a cell, thus providing one or more peptides of the invention to the class I MHC molecule binding pathway. Representative, but non-limiting examples of such approaches include the introduction of plasmid DNA through particle-mediated gene transfer or electroporation (Tuting, T. et al., J.Immunol., 160:1139-1147, (1998)), or the transduction of cells with an adenovirus expressing the polynucleotide of interest (Perez-Diez, A. et al., Cancer Res., 58:5305-5309, (1998)). Thus, oligonucleotides that code for one or more of the peptides of the invention can be provided to antigen presenting cells in such a fashion that the peptides associate with class I MHC molecules and are presented on the surface of the antigen presenting cell, and consequently are available to stimulate a CTL response.

By preparing the stimulator cells used to generate an in vitro CTL response in different ways, it is possible to control the peptide specificity of CTL response. For

example, the CTLs generated with a particular peptide will necessarily be specific for that peptide. Likewise, CTLs that are generated with a polypeptide or polynucleotide expressing or coding for particular peptides will be limited to specificities that recognize those peptides. More broadly, stimulator cells, and more specifically dendritic cells, can be incubated in the presence of the whole protein. As a further alternative, stimulator cells, and more specifically dendritic cells, can be transduced or transfected with RNA or DNA comprising the polynucleotide sequence encoding the protein. Under these alternative conditions, peptide epitopes that are naturally cleaved out of the protein, and which are generated in addition to peptide epitopes of SEQ ID NO:1 to 354 can associate with an appropriate class I MHC molecule, which may or may not include HLA-A1, -A2, or -A3. The selection of antigen presenting cells and the type of antigen with which to stimulate the CTL, is left to the ordinary skilled artisan.

In certain embodiments, the methods of the present invention include a method for inducing a CTL response in vitro that is specific for a tumor cell expressing a molecule from A1, A2, or A3 supertypes (A11 is a member of the A3 supertype), whereby the method comprises contacting a CTL precursor lymphocyte with an antigen presenting cell that has bound an immunogen comprising one or more of the peptides disclosed according to the invention.

In specific embodiments, the methods of the present invention include a method for inducing a CTL response in vitro that is specific for a tumor cell expressing a molecule from A1, A2, or A3 supertypes, whereby the method comprises contacting a CTL precursor lymphocyte with an antigen presenting cell that has exogenously acquired an immunogenic oligopeptide or polypeptide that comprises one or more of the peptides disclosed according to the invention.

A yet additional embodiment of the present invention is directed to a process for inducing a CTL response in vitro that is specific for a tumor cell expressing a molecule from A1, A2, or A3 supertypes, comprising contacting a CTL precursor lymphocyte with an antigen presenting cell that is expressing a polynucleotide coding for a polypeptide of the invention and wherein said polynucleotide is operably linked to a promoter.

In specific embodiments, the methods of the present invention include a method for inducing a CTL response in vitro that is specific for a tumor cell expressing HLA-A1, HLA-A2, or HLA-A11, whereby the method comprises contacting a CTL precursor lymphocyte with an antigen presenting cell that has bound an immunogen comprising one or more of the peptides disclosed according to the invention.

In specific embodiments, the methods of the present invention include a method for inducing a CTL response in vitro that is specific for a tumor cell expressing HLA-A1, HLA-A2, or HLA-A11, whereby the method comprises contacting a CTL precursor lymphocyte with an antigen presenting cell that has exogenously acquired an immunogenic oligopeptide or polypeptide that comprises one or more of the peptides disclosed according to the invention.

A yet additional embodiment of the present invention is directed to a process for inducing a CTL response in vitro that is specific for a tumor cell expressing HLA-A1, HLA-A2, or HLA-A11, comprising contacting a CTL precursor lymphocyte with an antigen presenting cell that is expressing a polynucleotide coding for a polypeptide of the invention and wherein said polynucleotide is operably linked to a promoter.

A variety of techniques exist for assaying the activity of CTL. These techniques include the labeling of target cells with radionuclides such as $Na_2{}^{51}CrO_4$ or ^{3}H-thymidine, and measuring the release or retention of the radionuclides from the target cells as an index of cell death. Such assays are well-known in the art and their selection is left to the skilled artisan. Alternatively, CTL are known to release a variety of cytokines when they are stimulated by an appropriate target cell, such as a tumor cell expressing the relevant class I MHC molecule and the corresponding peptide. Non-limiting examples of such cytokines include IFN-γ, TNFα, and GM-CSF. Assays for these cytokines are well known in the art, and their selection is left to the skilled artisan. Methodology for measuring both target cell death and cytokine release as a measure of CTL reactivity are given in (Coligan, J. E. et al, Current Protocols in Immunology, 1999, John Wiley & Sons, Inc., New York).

After expansion of the antigen-specific CTLs, the latter are then adoptively transferred back into the patient, where they will destroy their specific target cell. The utility of such adoptive transfer is demonstrated in North, R. J. et al. (Infect.Immun., 67:2010-2012, (1999)) and Riddell, S. R. et al. (Science, 257:238-241, (1992)). In determining the amount of cells to reinfuse, the skilled physician will be guided by the total number of cells available, the activity of the CTL as measured in vitro, and the condition of the patient. Preferably, however, about 1×10^6 to about 1×10^{12}, more preferably about 1×10^8 to about 1×10^{11}, and even more preferably, about 1×10^9 to about 1×10^{10} peptide-specific CTL are infused. Methodology for reinfusing the T cells into a patient are well known and exemplified in U.S. Pat. No. 4,844,893 to Honski, et al., and U.S. Pat. No. 4,690,915 to Rosenberg.

The peptide-specific CTL can be purified from the stimulator cells prior to infusion into the patient. For example, monoclonal antibodies directed towards the cell surface protein CD8, present on CTL, can be used in conjunction with a variety of isolation techniques such as antibody panning, flow cytometric sorting, and magnetic bead separation to purify the peptide-specific CTL away from any remaining non-peptide specific lymphocytes or from the stimulator cells. These methods are well known in the art, and are their selection is left to the skilled artisan. It should be appreciated that generation of peptide-specific CTL in this manner, obviates the need for stimulating the CTL in the presence of tumor. Thus, there is no chance of inadvertently reintroducing tumor cells into the patient.

Thus, one embodiment of the present invention relates to a process for treating a subject with cancer characterized by tumor cells expressing complexes of a molecule from A1, A2, or A3 supertypes, for example, HLA-A1, HLA-A2, or HLA-A11, whereby CTLs produced in vitro according to the present invention are administered in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines.

Another embodiment of the present invention is directed to a process for treating a subject with cancer characterized by tumor cells expressing any class I MHC molecule and an epitope of SEQ ID NO: 1 to 354, whereby the CTLs are produced in vitro and are specific for the epitope or original protein and are administered in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines.

In the foregoing embodiments the cancer to be treated may include a colorectal carcinoma, an ovarian carcinoma, a breast carcinoma, a lung carcinoma, and prostate carcinoma, but especially ovarian carcinoma.

The ex vivo generated CTL can be used to identify and isolate the T cell receptor molecules specific for the peptide. The genes encoding the alpha and beta chains of the T cell receptor can be cloned into an expression vector system and transferred and expressed in naive T cells from peripheral blood, T cells from lymph nodes, or T lymphocyte progenitor cells from bone marrow. These T cells, which would then be expressing a peptide-specific T cell receptor, would then have anti-tumor reactivity and could be used in adoptive therapy of cancer, and more specifically cancer, colorectal carcinoma, ovarian carcinoma, breast carcinoma, lung carcinoma, and prostate carcinoma.

In addition to their use for therapeutic or prophylactic purposes, the immunogenic peptides of the present invention are useful as screening and diagnostic agents. Thus, the immunogenic peptides of the present invention, together with modern techniques of gene screening, make it possible to screen patients for the presence of genes encoding such peptides on cells obtained by biopsy of tumors detected in such patients. The results of such screening may help determine the efficacy of proceeding with the regimen of treatment disclosed herein using the immunogens of the present invention.

Alternatively, the immunogenic peptides disclosed herein, as well as functionally similar homologs thereof, may be used to screen a sample for the presence of CTLs that specifically recognize the corresponding epitopes. The lymphocytes to be screened in this assay will normally be obtained from the peripheral blood, but lymphocytes can be obtained from other sources, including lymph nodes, spleen, tumors, and pleural fluid. The peptides of the present invention may then be used as a diagnostic tool to evaluate the efficacy of the immunotherapeutic treatments disclosed herein. Thus, the in vitro generation of CTL as described above would be used to determine if patients are likely to respond to the peptide in vivo. Similarly, the in vitro generation of CTL could be done with samples of lymphocytes obtained from the patient before and after treatment with the peptides. Successful generation of CTL in vivo should then be recognized by a correspondingly easier ability to generate peptide-specific CTL in vitro from lymphocytes obtained following treatment in comparison to those obtained before treatment.

The oligopeptides of the invention, such as SEQ ID NO: 1 to 354, can also be used to prepare class I MHC tetramers which can be used in conjunction with flow cytometry to quantitate the frequency of peptide-specific CTL that are present in a sample of lymphocytes from an individual. Specifically, for example, class I MHC molecules comprising peptides of SEQ ID NO: 1 to 354, would be combined to form tetramers as exemplified in U.S. Pat. No. 5,635,363. Said tetramers would find use in monitoring the frequency of CTLs in the peripheral blood, lymph nodes, or tumor mass of an individual undergoing immunotherapy with the peptides, proteins, or polynucleotides of the invention, and it would be expected that successful immunization would lead to an increase in the frequency of the peptide-specific CTL.

As stated above, a vaccine in accordance with the present invention may include one or more of the hereinabove described polypeptides or active fragments thereof, or a composition, or pool, of immunogenic peptides disclosed herein. When employing more than one polypeptide or active fragment, two or more polypeptides and/or active fragments

may be used as a physical mixture or as a fusion of two or more polypeptides or active fragments. The fusion fragment or fusion polypeptide may be produced, for example, by recombinant techniques or by the use of appropriate linkers for fusing previously prepared polypeptides or active fragments.

The immunogenic molecules of the invention, including vaccine compositions, may be utilized according to the present invention for purposes of preventing, suppressing or treating diseases causing the expression of the immunogenic peptides disclosed herein, such as where the antigen is being expressed by tumor cells. As used in accordance with the present invention, the term "prevention" relates to a process of prophylaxis in which an animal, especially a mammal, and most especially a human, is exposed to an immunogen of the present invention prior to the induction or onset of the disease process. This could be done where an individual has a genetic pedigree indicating a predisposition toward occurrence of the disease condition to be prevented. For example, this might be true of an individual whose ancestors show a predisposition toward certain types of cancer. Alternatively, the immunogen could be administered to the general population as is frequently done for infectious diseases. Alternatively, the term "suppression" is often used to describe a condition wherein the disease process has already begun but obvious symptoms of said condition have yet to be realized. Thus, the cells of an individual may have become cancerous but no outside signs of the disease have yet been clinically recognized. In either case, the term prophylaxis can be applied to encompass both prevention and suppression. Conversely, the term "treatment" is often utilized to mean the clinical application of agents to combat an already existing condition whose clinical presentation has already been realized in a patient. This would occur where an individual has already been diagnosed as having a tumor.

It is understood that the suitable dosage of an immunogen of the present invention will depend upon the age, sex, health, and weight of the recipient, the kind of concurrent treatment, if any, the frequency of treatment, and the nature of the effect desired. However, the most preferred dosage can be tailored to the individual subject, as determined by the researcher or clinician. The total dose required for any given treatment will commonly be determined with respect to a standard reference dose as set by a manufacturer, such as is commonly done with vaccines, such dose being administered either in a single treatment or in a series of doses, the success of which will depend on the production of a desired immunological result (i.e., successful production of a CTL-mediated response to the antigen, which response gives rise to the prevention and/or treatment desired). Thus, the

overall administration schedule must be considered in determining the success of a course of treatment and not whether a single dose, given in isolation, would or would not produce the desired immunologically therapeutic result or effect.

The therapeutically effective amount of a composition containing one or more of the immunogens of this invention, is an amount sufficient to induce an effective CTL response to the antigen and to cure or arrest disease progression. Thus, this dose will depend, among other things, on the identity of the immunogens used, the nature of the disease condition, the severity of the disease condition, the extent of any need to prevent such a condition where it has not already been detected, the manner of administration dictated by the situation requiring such administration, the weight and state of health of the individual receiving such administration, and the sound judgment of the clinician or researcher. Thus, for purposes of prophylactic or therapeutic administration, effective amounts would generally lie within the range of from 1.0 μg to about 5,000 μg of peptide for a 70 kg patient, followed by boosting dosages of from about 1.0 μg to about 1,000 μg of peptide pursuant to a boosting regimen over days, weeks or even months, depending on the recipient's response and as necessitated by subsequent monitoring of CTL-mediated activity within the bloodstream. Of course, such dosages are to be considered only a general guide and, in a given situation, may greatly exceed such suggested dosage regimens where the clinician believes that the recipient's condition warrants more a aggressive administration schedule. Needless to say, the efficacy of administering additional doses, and of increasing or decreasing the interval, may be re-evaluated on a continuing basis, in view of the recipient's immunocompetence (for example, the level of CTL activity with respect to tumor-associated or tumor-specific antigens).

For such purposes, the immunogenic compositions according to the present invention may be used against a disease condition such as cancer by administration to an individual by a variety of routes. The composition may be administered parenterally or orally, and, if parenterally, either systemically or topically. Parenteral routes include subcutaneous, intravenous, intradermal, intramuscular, intraperitoneal, intranasal, transdermal, or buccal routes. One or more such routes may be employed. Parenteral administration can be, for example, by bolus injection or by gradual perfusion over time.

Generally, vaccines are prepared as injectables, in the form of aqueous solutions or suspensions. Vaccines in an oil base are also well known such as for inhaling. Solid forms which are dissolved or suspended prior to use may also be formulated. Pharmaceutical carriers, diluents and excipients are generally added that are compatible with the active

ingredients and acceptable for pharmaceutical use. Examples of such carriers include, but are not limited to, water, saline solutions, dextrose, or glycerol. Combinations of carriers may also be used. These compositions may be sterilized by conventional, well known sterilization techniques including sterile filtration. The resulting solutions may be packaged for use as is, or the aqueous solutions may be lyophilized, the lyophilized preparation being combined with sterile water before administration. Vaccine compositions may further incorporate additional substances to stabilize pH, or to function as adjuvants, wetting agents, or emulsifying agents, which can serve to improve the effectiveness of the vaccine.

The concentration of the CTL stimulatory peptides of the invention in pharmaceutical formulations are subject to wide variation, including anywhere from less than 0.01% by weight to as much as 50% or more. Factors such as volume and viscosity of the resulting composition must also be considered. The solvents, or diluents, used for such compositions include water, possibly PBS (phosphate buffered saline), or saline itself, or other possible carriers or excipients.

The immunogens of the present invention may also be contained in artificially created structures such as liposomes, ISCOMS, slow-releasing particles, and other vehicles which increase the immunogenicity and/or half-life of the peptides or polypeptides in serum. Liposomes include emulsions, foams, micelles, insoluble monolayers, liquid crystals, phospholipid dispersions, lamellar layers and the like. Liposomes for use in the invention are formed from standard vesicle-forming lipids which generally include neutral and negatively charged phospholipids and a sterol, such as cholesterol. The selection of lipids is generally determined by considerations such as liposome size and stability in the blood. A variety of methods are available for preparing liposomes as reviewed, for example, by (Coligan, J. E. et al, Current Protocols in Protein Science, 1999, John Wiley & Sons, Inc., New York)and see also U.S. Pat. Nos. 4,235,871, 4,501,728, 4,837,028, and 5,019,369.

Liposomes containing the peptides or polypeptides of the invention can be directed to the site of lymphoid cells where the liposomes then deliver the selected immunogens directly to antigen presenting cells. Targeting can be achieved by incorporating additional molecules such as proteins or polysaccharides into the outer membranes of said structures, thus resulting in the delivery of the structures to particular areas of the body, or to particular cells within a given organ or tissue. Such targeting molecules may a molecule that binds to receptor on antigen presenting cells. For example an antibody that binds to CD80 could be used to direct liposomes to dendritic cells.

The immunogens of the present invention may also be administered as solid compositions. Conventional nontoxic solid carriers including pharmaceutical grades of mannitol, lactose, starch, magnesium, cellulose, glucose, sucrose, sodium saccharin, and the like. Such solid compositions will often be administered orally, whereby a pharmaceutically acceptable nontoxic composition is formed by incorporating the peptides and polypeptides of the invention with any of the carriers listed above. Generally, such compositions will contain 10-95% active ingredient, and more preferably 25-75% active ingredient.

Aerosol administration is also an alternative, requiring only that the immunogens be properly dispersed within the aerosol propellant. Typical percentages of the peptides or polypeptides of the invention are 0.01%-20% by weight, preferably 1% -10%. The use of a surfactant to properly disperse the immunogen may be required. Representative surfactants include the esters or partial esters of fatty acids containing from 6 to 22 carbon atoms, such as caproic, octanoic, lauric, palmitic, stearic, linoleic, linolenic, olesteric and oleic acids with an aliphatic polyhydric alcohol or its cyclic anhydride. Mixed esters, such as mixed or natural glycerides may be employed. The surfactant may constitute 0.1-20% by weight of the composition, preferably 0.25-5%. Typical propellants for such administration may include esters and similar chemicals but are by no means limited to these. A carrier, such as lecithin for intranasal delivery, may also be included.

The peptides and polypeptides of the invention may also be delivered with an adjuvant. Adjuvants include, but are not limited to complete or incomplete Freund's adjuvant, Montanide ISA-51, Lymphocyte Activation Gene-3 (LAG-)3, aluminum phosphate, aluminum hydroxide, alum, and saponin. Adjuvant effects can also be obtained by injecting a variety of cytokines along with the immunogens of the invention. These cytokines include, but are not limited to IL-1, IL-2, IL-7, IL-12, and GM-CSF.

The peptides and polypeptides of the invention can also be added to professional antigen presenting cells such as dendritic cells that have been prepared ex vivo. For example, the dendritic cells could be prepared from CD34 positive stem cells from the bone marrow, or they could be prepared from CD14 positive monocytes obtained from the peripheral blood. The dendritic cells are generated ex vivo using cytokines such as GM-CSF, IL-3, IL-4, TNF, and SCF. The cultured DC are then pulsed with peptides at various concentrations using standard methods that are well known in the art. The peptide-pulsed dendritic cells can then be administered either intraveneously, subcutaneously, or intradermally, and the immunization may also include cytokines such as IL-2 or IL-12.

The present invention is also directed to a vaccine in which an immunogen of the present invention is delivered or administered in the form of a polynucleotide encoding the a polypeptide or active fragment as disclosed herein, whereby the peptide or polypeptide or active fragment is produced in vivo. The polynucleotide may be included in a suitable expression vector and combined with a pharmaceutically acceptable carrier. For example, the peptides or polypeptides could be expressed in plasmid DNA and nonreplicative viral vectors such as vaccinia, fowlpox, Venezuelan equine encephalitis virus, adenovirus, or other RNA or DNA viruses. These examples are meant to be illustrative only and should not be viewed as self-limiting A wide variety of other vectors are available and are apparent to those skilled in the art from the description given herein. In this approach, a portion of the nucleotide sequence of the viral vector is engineered to express the peptides or polypeptides of the invention. Vaccinia vectors and methods useful in immunization protocols are described in U.S. Pat. No. 4,722,848, the disclosure of which is incorporated herein by reference in its entirety.

Regardless of the nature of the composition given, additional therapeutic agents may also accompany the immunogens of the present invention. Thus, for purposes of treating tumors, compositions containing the immunogens disclosed herein may, in addition, contain other antitumor pharmaceuticals. The use of such compositions with multiple active ingredients is left to the discretion of the clinician.

In addition, the immunogens of the present invention can be used to stimulate the production of antibodies for use in passive immunotherapy, for use as diagnostic reagents, and for use as reagents in other processes such as affinity chromatography.

The present invention also relates to antibodies that react with immunogens, such as a polypeptide comprising one or more of the epitopic peptides of SEQ ID NO: 1 to 354 as disclosed herein. Active fragments of such antibodies are also specifically contemplated. Such antibodies, and active fragments of such antibodies, for example, and Fab structure, may react with, including where it is highly selective or specific for, an immunogenic structure comprising 2, 3, 4 or more of the epitopic peptides of the invention.

With the advent of methods of molecular biology and recombinant technology, it is now possible to produce antibody molecules by recombinant means and thereby generate gene sequences that code for specific amino acid sequences found in the polypeptide structure of the antibodies. Such antibodies can be produced by either cloning the gene sequences encoding the polypeptide chains of said antibodies or by direct synthesis of said polypeptide chains, with in vitro assembly of the synthesized chains to form active

tetrameric (H_2L_2) structures with affinity for specific epitopes and antigenic determinants. This has permitted the ready production of antibodies having sequences characteristic of neutralizing antibodies from different species and sources.

Regardless of the source of the antibodies, or how they are recombinantly constructed, or how they are synthesized, in vitro or in vivo, using transgenic animals, such as cows, goats and sheep, using large cell cultures of laboratory or commercial size, in bioreactors or by direct chemical synthesis employing no living organisms at any stage of the process, all antibodies have a similar overall 3 dimensional structure. This structure is often given as H_2L_2 and refers to the fact that antibodies commonly comprise 2 light (L) amino acid chains and 2 heavy (H) amino acid chains. Both chains have regions capable of interacting with a structurally complementary antigenic target. The regions interacting with the target are referred to as "variable" or "V" regions and are characterized by differences in amino acid sequence from antibodies of different antigenic specificity.

The variable regions of either H or L chains contains the amino acid sequences capable of specifically binding to antigenic targets. Within these sequences are smaller sequences dubbed "hypervariable" because of their extreme variability between antibodies of differing specificity. Such hypervariable regions are also referred to as "complementarity determining regions" or "CDR" regions. These CDR regions account for the basic specificity of the antibody for a particular antigenic determinant structure.

The CDRs represent non-contiguous stretches of amino acids within the variable regions but, regardless of species, the positional locations of these critical amino acid sequences within the variable heavy and light chain regions have been found to have similar locations within the amino acid sequences of the variable chains. The variable heavy and light chains of all antibodies each have 3 CDR regions, each non-contiguous with the others (termed L1, L2, L3, H1, H2, H3) for the respective light (L) and heavy (H) chains. The accepted CDR regions have been described in the text and figures of Kabat et al. (J. Biol. Chem. 252:6609-6616 (1977)).

In all mammalian species, antibody polypeptides contain constant (i.e., highly conserved) and variable regions, and, within the latter, there are the CDRs and the so-called "framework regions" made up of amino acid sequences within the variable region of the heavy or light chain but outside the CDRs.

The antibodies disclosed according to the invention may also be wholly synthetic, wherein the polypeptide chains of the antibodies are synthesized and, possibly, optimized for binding to the polypeptides disclosed herein as being receptors. Such antibodies may be

chimeric or humanized antibodies and may be fully tetrameric in structure, or may be dimeric and comprise only a single heavy and a single light chain. Such antibodies may also include fragments, such as Fab and $F(ab_2)'$ fragments, capable of reacting with and binding to any of the polypeptides disclosed herein as being receptors.

A further embodiment of the present invention relates to a method for inducing a CTL response in a subject comprising administering to subjects that express HLA-A1 antigens an effective (i.e., CTL-stimulating amount) of an immunogen of the invention that does not comprise the entire protein expressing the epitopic peptides disclosed herein (i.e., one that comprises less than the entire protein where the protein is a naturally occurring polypeptide) in an amount sufficient to induce a CTL response to tumor cells expressing at least HLA-A1 or HLA-A2, as the case may be, thereby eliciting a cellular response against said tumor cells.

A still further embodiment of the present invention relates to a method for inducing a CTL response in a subject, wherein the immunogen is in the form of a polynucleotide. In one non-limiting example, the method comprises administering to subjects that express HLA-A1 at least one CTL epitope, wherein said epitope or epitopes are selected from a group comprising the peptides disclosed according to the invention, and are coded within a polynucleotide sequence that does not comprise the entire protein coding region, in an amount sufficient to induce a CTL response to tumor cells expressing HLA-A1 or HLA-A2.

While the below examples are provided to illustrate the invention, it is to be understood that these methods and examples in no way limit the invention to the embodiments described herein and that other embodiments and uses will no doubt suggest themselves to those skilled in the art. All publications, patents, and patent applications cited herein are hereby incorporated by reference, as are the references cited therein. It is also to be understood that throughout this disclosure where the singular is used, the plural may be inferred and vice versa and use of either is not to be considered limiting.

Example 1

Cell Lines

For HLA-A1 and HLA-A11 studies, ARGOV57, a HLA-A1/11 positive ovarian cell line, was established by culturing tumor cells from an ascitic fluid from an ovarian patient.

For HLA-A2 studies, OVCAR3, a HLA-A2 positive ovarian carcinoma cell line, was established by culturing tumor cells from an ascitic fluid from an ovarian patient.

SKOV3-A2, a HLA-A2 stably expressing ovarian carcinoma cell line, was established by culturing tumor cells from an ascitic fluid from an ovarian patient and transduced with HLA-A2 gene.

Example 2

Immunoaffinity Purification

ARGOV57 cells were grown in 10-chamber Nunc cell factories (Fisher, Pittsburgh, Pa.). The cells were harvested by treatment with 0.45% trypsin and 0.32 mM EDTA, washed two times in phosphate-buffered saline solution (pH 7.4), and stored as cell pellets at -80° C. Aliquots of 6-8 X 10^{10} cells were solubilized at 5-10 X 10^{6} cells/ml in 20 mM Tris, pH 8.0, 150 mM NaCl, 1% CHAPS, 18.5 µg/ml iodoacetamide, 5 µg/ml aprotonin, 10 µg/ml leupeptin, 10 µg/ml pepstatin A, 5 mM EDTA, 0.2% sodium azide, and 17.4 µg/ml phenylmethylsulfonyl fluoride for 1 h. This and all subsequent steps were performed with ice-cold solutions and at 4° C. The lysates were then centrifuged at 100,000 X g, the pellets discarded, and the supernatants passed through a 0.22 µm filter. The supernatants were then passed over a series of columns with the first containing Sepharose, and the second containing the HLA-A1-specific monoclonal antibody, GAP-A1, bound to a protein A-Sepharose matrix. The second column was then sequentially washed with 20 column volumes of 20 mM Tris, pH 8.0, 150 mM NaCl, 20 column volumes of 20 mM Tris, pH 8.0, 1.0 M NaCl, and 20 column volumes of 20 mM Tris, pH 8.0. The peptides were eluted from the column with 5 column volumes of 10% acetic acid. The isolated HLA-A1 molecules were then boiled for 5 min to further dissociate any bound peptide from the heavy chains. The peptides were then separated from the co-purifying class I heavy chain and β_2-microglobulin by centrifugation on a Ultrafree-CL membrane with a nominal molecular weight cut-off of 5,000 Daltons (Millipore, Bedford, Mass.).

For a separate study, OVCAR3 or SKOV3 cells were successfully prepared using the same procedure as just described except that HLA-A2 molecules were prepared using HLA-A2 specific antibodies.

Example 3

Peptide Fractionation

The peptide extracts were fractionated by RP-HPLC (Reversed Phase -High Performance Liquid Chromatography) using an Applied Biosystems (ABI) model 140B

system. The extracts were concentrated by vacuum centrifugation from about 20 ml down to 250 µl and injected into either a Brownlee (Norwalk, Conn.) C_{18} Aquapore column (2.1 mm X 3 cm; 300 Å; 7 µm) or a Higgins (Mountain View, Calif.) C18 Haisil column (2.1 mm X 4 cm; 300 Å; 5 µm). The peptides were eluted by first using a gradient of acetonitrile/0.085% TFA (trifluoroacetic acid) in 0.1% TFA/water, with the concentration of acetonitrile increasing from 0-9% (0-5 minutes), 9-36% (5-55 minutes), and 36-60% (55-62 minutes). A second dimension fractionation of combined fractions 17 and 18 from the first dimension (TFA) fraction was accomplished using the same gradient but with the substitution of HFBA (heptafluorobutyric acid) for TFA. The flow rate was 200 µl/min, and fractions were collected at 1 min (Brownlee column) or 40 second (Higgins column) intervals. A third dimension of RP-HPLC was achieved using an Eldex (Napa, Calif.) MicroPro Pump, a homemade C_{18} microcapillary column, and an ABI model 785A UV absorbance detector. The column was prepared by packing a 27 cm bed of 10 µm C_{18} particles in a section of 285 µm o.d./75 µm i.d. fused silica (Polymicro Technologies, Phoenix, Ariz.). Peptides in combined fractions 26 and 27 of the second dimension fraction were loaded onto this column and eluted with a gradient of acetonitrile/0.67% triethylamine acetate/water in 0.1% triethylamine acetate/water, with the concentration of acetonitrile increasing from 0-60% in 40 minutes. The flow rate was about 300 nl/min, and fractions were collected into 25 µl of water every 30 s. In all RP-HPLC experiments, peptides were detected by monitoring UV absorbance at 214 nm.

Example 4

Mass Spectrometric Analysis

The second dimension HPLC fraction was analyzed using an affluent splitter on the microcapillary HPLC column. In this experiment, the column (360 µm o.d. X 100 µm i.d. with a 25 cm C_{18} bed) was butt connected with a zero dead volume tee (Valco, Houston, Tex.) to two pieces of fused silica of different lengths (25 µm and 40 µm i.d.). Peptides were eluted with a 34 min gradient of 0-60% acetonitrile. The 25 µm capillary deposited one-fifth of the HPLC effluent into the wells of a microtiter plate for use in CTL epitope reconstitution assays, whereas the remaining four-fifths of the effluent was directed into the mass spectrometer. Ions were formed by electrospray ionization, and mass spectra were recorded by scanning between mass to charge ratios (m/z) 300 and 1400 every 1.5 seconds. Peptide sequences were determined by CAD (collision-activated dissociation) tandem mass

spectrometry as described in the literature (Hunt, D. F. et al., Proc. Natl. Acad. Sci. U.S.A, 83:6233-6237, (1986)).

Example 5

Homology searches of identified peptide sequences

Proteins containing peptides corresponding to the masses identified by MS were analyzed with the search algorithm, SEQUEST. Searches were also carried out on the GenBank non-redundant sequence database (http://www.ncbi.nlm.nih.gov/Entrez/) as well as on our own unique database of 2943 specific sequences compiled from GenBank and EST data-base entries. Upon experimental confirmation of the peptide sequence, a tBLASTn search of the GenBank non-redundant database was performed to identify any genes containing the DNA sequence encoding the peptide.

Example 6

Peptide Synthesis

Peptides were synthesized using a Gilson (Madison, Wis.) AMS 422 multiple peptide synthesizer. Quantities of 10 μMol were synthesized using conventional FMOC amino acids, resins, and chemical techniques. Peptides were purified by RP-HPLC using a 4.6 mm X 100 mm POROS (Perseptive Biosystems, Cambridge, Mass.) column and a 10 min, 0-60% acetonitrile in 0.1% TFA gradient.

Example 7

Generation of monocyte-derived DC and peptide loading

PBMC were purified from HLA-$A2^+$ normal donor blood using lymphocyte separation media (Cappel ICN Biomedical, Aurora, OH). PBMC (5.3×10^6) were added to individual wells of a 24-well cluster plate (Costar, Corning, NY) in 1.0 ml of serum-free AIM-V medium (Life Technologies) and allowed to adhere for 60 min at 37°C. Non-adherent cells were removed and saved as a source of effector T cells. Adherent PBMC (~8.3×10^5/well) were then pulsed with 50 mg/ml synthetic peptides in serum-free AIM-V medium containing 1.5 mg/ml β_2-microg lobulin (Calbiochem-Novabiochem, San Diego, CA) and incubated for 2 h at 37°C. Unbound peptides were aspirated and the wells washed with media.

Monocyte-derived DC were generated as follows. PBMC (5.3×10^7) were allowed to adhere in T-75 flasks (Corning) in 10 ml of serum-free AIM-V medium for 60 min at 37°C. Non-adherent cells were collected as a source of effector T cells and pooled with the previous collection above. Adherent monocytes in flasks were then exposed to recombinant human granulocyte macrophage colony stimulating factor (GM-CSF, 25 ng/ml; Peprotech) and recombinant human IL-4 (100 ng/ml; Peprotech) in 10 ml of AIM-V medium containing 10% heat-inactivated FBS. DC obtained by this method [immature DC (iDC)] are characterized by expression of low levels of CD83, CD80, CD86, and HLA class I and class II molecules (data not shown).

Mature DC (mDC) were obtained by exposing day 5 DC cultures to recombinant soluble CD40 ligand (sCD40L; Peprotech) at 1.5 mg/ml for 24 h in the presence of 25 ng/ml GM-CSF and are characterized by expression of high levels of CD80, CD86, and HLA class I and class II molecules. mDC were harvested, washed, pulsed with 5 mg/ml peptide in serum-free AIM-V medium and irradiated (5000 rad) prior to use as stimulators.

Example 8

Generation of peptide-specific CTL

The protocol used here is a modification of the method described by Plebanski et al. (Eur. J. Immunol. 25:1783, (1995)). CTL to peptide were generated by 3±4 cycles of stimulation with peptide-loaded APC. For the first round of stimulation (day 0), T cells or non-adherent PBMC from above (2.3×10^6/ml or 4.3×10^6 per well) were added in bulk ($CD4^+$, $CD8^+$, NK, etc.) to adherent PBMC-loaded peptides in serum-free medium (50 mg/ml), β_2-microglobulin (1.5 mg/ml) (Calbiochem-Novabiochem), recombinant human IL-7 (5 ng/ml) (Peprotech) and keyhole limpet hemocyanin (5 mg/ml) (Sigma, St Louis, MO). Cultures were re-stimulated with iDC every 7 days, pulsed with varying amounts of peptide (second round 25 mg/ml, third round 10 mg/ml) and irradiated (5000 rad) on day 8. At each re-stimulation, the T cells were transferred to new plates by first aspirating 70% of spent media in wells and then transferring the pooled contents to a new plate. Fresh IL-7 was added at each re-stimulation. The responder:stimulator (T cell:DC) ratio was set at 20 for each stimulation. Recombinant human IL-2 (10 U/ml) was added on day 5 after each re-stimulation.

Prior to ^{51}Cr-release assay, the T cells were harvested and $CD8^+$ T cells were purified by positive selection using $CD8^+$ microbeads immunomagnetic cell separation

with MACS kit (Miltenyi Biotec, Auburn, CA). If a fourth round of stimulation was necessary following CTL analysis, the CTL were pulsed as before, except with 5±10 mg/ml of peptide.

Example 9

Generation of allospecific CTL

HLA-A2-allospecific CTL were obtained in a mixed lymphocyte reaction by repeated stimulation of HLA-A3$^+$ PBMC (responders) with irradiated HLA-A2$^+$ stimulator PBMC at a ratio of 10:1 in the presence of 10 U/ml IL-2. Stimulation was repeated weekly with PBMC from different HLA-A2$^+$ donors so as to minimize alloresponse to non-HLA-A2 antigens. T cells were assessed for lysis on several HLA-A2$^+$ targets including tumor cells, EBV-B cells and HLA-A3$^+$ targets every week after the third round of stimulation.

Example 10

CTL expansion

Expansion of large numbers of peptide-specific or HLA-A2-allospecific CTL was achieved by culturing 5.3 X 10^4±1.3 X 10^5 T cells around day 6 or 7 post peptide- or allostimulation in the presence of 2.5-3.0 X 10^7 irradiated (5000 rad) allogeneic normal donor PBMC coated with anti-CD3 antibody at 10 ng/ml (BD PharMingen, San Diego, CA) and 25 U/ml of recombinant human IL-2 (Peprotech) in a final volume of 30 ml RPMI medium. Media changes with IL-2 addition (50 U/ml) were effected on days 5 and 8. Cells were harvested for cytotoxicity assays on days 10±12 and re-stimulated or frozen for later use.

Example 11

^{51}Cr-release cytotoxicity assay

The standard 4-h Cr-release assay was performed in 96-well V-bottomed microplates. Target cells in suspension (T2, C1R.A2, B-LCL and K562) were labeled with 100 mCi $Na_2{}^{51}CrO4$ (NEN Life Science, Boston, MA) per 1.3 X 10^6 cells either overnight (~ 6±18 h) in 5 ml RPMI 1640 media containing 2±5% FBS or for 60±90 min at 37°C directly with the cell pellet in the case of adherent cells (tumor cell lines and control lines). Labeling was terminated by washing the targets with cold media containing 5% FBS for a total of three washes. Target cells were resuspended at a concentration of 2-3 X 10^4/ml.

About 2-3 X 10^3 targets in 100 ml were delivered to each well containing CTL (effectors) seeded at different E:T ratios. Spontaneous release wells contained targets in media alone, while maximal release wells contained targets in 2% NP-40 detergent (Igepal CA-630; Sigma). HLA restriction of CTL-mediated killing was achieved by preincubation of targets with HLA-specific antibodies prior to incubation with CTL.

The plate was gently spun for 1±2 min and incubated at 37°C for 4 h. For harvesting assay plates, 100 ml of supernatants from the wells was transferred to counting tubes (USA Scientific) and g counts were determined in a g counter (ICN Micromedic Systems, Huntsville, AL). Cytolytic activity of T cells was expressed in percent specific lysis as follows: specific lysis = {[experimental release (c.p.m.) ± spontaneous release (c.p.m.)]/[maximal release (c.p.m.) ± spontaneous release (c.p.m.)]}.

Example 12

Competitive inhibition assay

Peptide-stimulated CTL were reacted with ^{51}Cr-labeled Ov2 tumor cells (E:T ratio of 40) in the presence of excess of cold targets in a 4-h Cr-release assay. Cold targets were either empty T2 cells, T2 cells pulsed with 1 mg/ml relevant peptide (used to stimulate CTL) or irrelevant (control) peptides (HER-2/neu 369±377 or MART 127±35), or IFN-γ pre-treated tumor cells (SKOV3.A2 and OVCAR 3) with the cold target in 5-fold excess of the hot target. Results indicate that (i) CTL show specific interaction with the peptide to which they are sensitized to, and (ii) CTL compete for similar epitopes presented on Ov2 as were found in SKOV3.A2 and OVCAR3 cell lines by MS.

Table 2. Description of Fragments, Parent Sequence Identification and Parent SwissProt Identification Number for peptides 1 to 354.

SEQ ID NO.	Fragment	Parent Protein Identification	SwissProt Id No.
1	AEAEFYRQV	BCL-6 corepressor long isoform_	Q6W2J9
2	KEFDGKSLV	Heat shock protein HSP 90-beta (HSP 84)	P08238
3	SLAEGLRTV	2'-5'oligoadenylate synthetase 3	Q2HJ14
4	YLGDGPKLV	26S protease regulatory subunit 4 (P26s4)	P62191
5	YLASLIRSV	26S proteasome non-ATPase regulatory subunit 7	P51665
6	KLLEPVLLL	40S ribosomal protein S16	P62249
7	KLIEVDDERKL	40S ribosomal protein S6 (Phosphoprotein NP33)	P62753
8	RLFEGNALL	40S ribosomal protein S9	P46781
9	TLYEAVREV	60S ribosomal protein L10a (CSA-19)	P62906
10	SLIKQIPRI	60S ribosomal protein L10a (CSA-19)	P62906
11	YLNDLIHSV	A kinase anchor protein 10, mitochondrial precursor	O43572
12	DVLKIPVQLV	Activated T-cell marker CD109	Q6YHK3
13	LSDPLKANV	Activin receptor type 2A precursor (EC 2.7.11.30)	P27037
14	DLCFEKVNV	ADAM19 protein	Q8TBU7
15	KLHDINAQL	AP-1 complex subunit beta-1	Q10567
16	IDAIRIPVL	Lung alpha/beta hydrolase protein 1	Q96SE0
17	FIASKGVKLV	Alpha-actinin-3	Q08043
18	KIVKRPSLQFL	Ankyrin repeat and SOCS box protein 17	Q8WXJ9
19	TLVTVSAAKT	Anti-colorectal carcinoma heavy chain	Q65ZQ1
20	KVLDGSPIEV	APOBEC1 complementation factor	Q9NQ94
21	FLAEHPNVTL	Probable DNA dC->dU-editing enzyme APOBEC-3D	Q96AK3
22	NLVQDSLDL	Apolipoprotein-L4 precursor (Apolipoprotein L-IV)	Q9BPW4
23	ISENEKLQK	Apoptosis stimulating of p53 protein 1	Q96KQ4
24	VLAARNPAKV	Nucleoporin 188kDa (arachin)	Q5SRE5
25	RYFDGNLEKL	Protein ariadne-1 homolog (ARI-1)	Q9Y4X5
26	TLADVLYHV	Set1/Ash2 histone methyltransferase complex subunit ASH2	Q9UBL3
27	SYVLKKAQV	Ubiquitin carboxyl-terminal hydrolase 20 (EC 3.1.2.15)	Q9Y2K6
28	TVLLRLGDEL	Bcl-2 related ovarian killer	Q9UL32
29	QILLDETLK	Cell growth inhibiting protein 39	Q2TTR2
30	DECITNLLV	BH3-interacting domain death agonist (BID)	P55957
31	SLDERPVAV	Bone morphogenetic protein receptor type-2	Q13873

		precursor	
32	SLLLLPEKN	BRCA1 associated RING domain 1 variant	Q53F80
33	VLCVSDIISL	Breast cancer type 2 susceptibility protein	P51587
34	FLPDPSALQNL	BRCA1/BRCA2-containing complex subunit 45	Q9NXR7
35	MLNEHDFEV	Breast cancer 1 early onset	Q3LRJ0
36	VNTDFSPYL	Breast cancer 1 early onset	Q3LRJ0
37	EFMLVYKFAR	Breast and ovarian cancer susceptibility protein	Q7KYU6
38	TLWVDPYEV	BTG2 protein (NGF-inducible anti-proliferative protein PC3)	P78543
39	FLDHILASV	Nuclear protein 5qNCA	Q7LBC6
40	SLSMVNHRL	Integrin alpha-3 precursor (Galactoprotein B3)	P26006
41	RVDFPGFVR	Hepatocellular carcinoma- associated antigen 520	O43745
42	WTNPQFKI	Calpain-11 (EC 3.4.22.-)	Q9UMQ6
43	KIDPLEVEE	Neural cell adhesion molecule variant	Q59FY0
44	KLPEKWESV	Ribosomal L1 domain-containing protein 1	O76021
45	LIEKEKVLN	CENP-F kinetochore protein (Centromere protein F)	P49454
46	FLKEHMDEV	Pericentriol material 1	Q15154
47	KLLGELHTL	Pericentriol material 1	Q15154
48	TLVEAFPTL	Cervical cancer suppressor gene 5	Q8NFX8
49	GLGAEIEIR	Vacuolar protein sorting 13A	Q96RL7
50	GKLILLDKL	Chromodomain-helicase-DNA-binding protein 2	O14647
51	TTITVSPFY	Adiponutrin (iPLA2-epsilon)	Q9NST1
52	SILNEGGIK	CUB and sushi domain-containing protein 3 precursor	Q7Z407
53	YMADRLLGV	Cullin-7 (CUL-7)	Q14999
54	YLKDLIEEV	Cyclic AMP-dependent transcription factor ATF-4	P18848
55	YLDIKGLLD	Cyclin A/CDK2-associated protein p19	P63208
56	PCLSELIHKA	Cyclin-A1	P78396
57	TVLDFGVLASI	Cyclin M3, isoform 1	Q8NE01
58	QPLLKQSPW	CPEB2 protein	Q3B8N6
59	YLLPAIVHI	Probable ATP-dependent RNA helicase DDX5	P17844
60	KLLPGDIHQI	Dedicator of cytokinesis protein 1	Q14185
61	SLLKGDLKGV	Development and differentiation-enhancing factor 2	O43150
62	QLIDLSSPLI	G2 and S phase expressed protein 1	Q9NYZ3
63	PSPQLWTV	ATP-dependent helicase PRIC285	Q9BYK8
64	NMYGKVVTV	Transcription elongation factor SPT5 (DLC-1)	O00267
65	RLYDGLFKV	DNA damage-binding protein 1	Q16531
66	QNFVDSKEV	DNA excision repair protein ERCC-6	Q03468
67	ALIEKLVEL	DNA polymerase alpha subunit B	Q14181
68	VIEDDVNMAIR	DNA replication licensing factor MCM2	P49736
69	SQDEIKQEV	DNA2-like homolog	P51530
70	HLNGSCHLLI	Estrogen response element binding protein	O77798

71	ALIDRMVNL	DNA damage-inducible transcript 3 (DDIT-3)	P35638
72	SQKIQEAVKA	DNA-directed RNA polymerase I largest subunit	O95602
73	VLLGKVYVV	DRE1 protein	Q9NXT9
74	TIDELKEQV	Dynactin-1 (150 kDa dynein-associated polypeptide)	Q14203
75	SEVEQYVKY	Dynein heavy chain, cytosolic (DYHC)	Q14204
76	ETQLTYRR	Echinoderm microtubule associated protein-like 5	Q6UYC9
77	IKDDLEDLI	ECT2 protein (Epithelial cell-transforming sequence 2 oncogene)	Q9H8V3
78	QVLGKIERA	Endothelial differentiation-related factor 1 (EDF-1)	O60869
79	IQINLQRKM	Developmentally-regulated endothelial cell locus 1 protein	O43854
80	ALQEMVHQV	Enhancer of filamentation 1 (HEF1)	Q14511
81	SMYGVDLHHA	Band 4.1-like protein 3 (4.1B)	Q9Y2J2
82	SEDITRYYL	Band 4.1-like protein 3 (4.1B)	Q9Y2J2
83	SKEEDPFNV	Epidermal growth factor receptor substrate 15	P42566
84	FLDKQGFYV	Epidermal growth factor receptor substrate 15	P42566
85	AVQVLMVLSL	Epithelial membrane protein 3 (EMP-3) (YMP protein)	P54852
86	FLSHKLDIK	Protocadherin Fat 2 precursor (hFat2)	Q9NYQ8
87	VEPALRKPP	Protocadherin Fat 2 precursor (hFat2)	Q9NYQ8
88	YLNKLLITR	Fibroblast growth factor receptor-like 1 precursor	Q8N441
89	YLLDVLERS	Fibroblast growth factor receptor 4 precursor	P22455
90	TASPDYLEI	Fibroblast growth factor receptor 2 precursor	P21802
91	EQSLETTKV	FKSG73	Q9BWW1
92	SLFPGKLEV	Protein flightless-1 homolog	Q13045
93	DVGKDQEFTV	Filamin-A (Alpha-filamin) (Filamin-1)	P21333
94	KIMTEKELLAV	Flotillin-2 (Epidermal surface antigen) (ESA)	Q14254
95	SLLESVQKL	Serine/threonine-protein kinase ATR	Q13535
96	YLQPKLLGI	Serine/threonine-protein kinase ATR	Q13535
97	YLLVGTLFLL	Frizzled 5 precursor (Frizzled-5)	Q13467
98	MELSEPIVEN	G1 to S phase transition protein 1 homolog	P15170
99	QLVVELKDI	Golgin subfamily B member 1 (Giantin)	Q14789
100	YIDDHSWTL	Growth factor receptor-bound protein 14	Q14449
101	SLYEENNKL	GRIP and coiled-coil domain-containing protein 2	Q8IWJ2
102	KLLEVQILE	GRIP and coiled-coil domain-containing protein 2	Q8IWJ2
103	YIDDVFHAL	GTP-binding protein Rif1	Q92963
104	ALKDKIEKA	Tumor rejection antigen (Gp96) 1	Q5CAQ5
105	KIILRHLIE	Heat-shock protein beta-3 (HspB3)	Q12988
106	TLGKLFWV	Low-density lipoprotein receptor-related protein 5	O75197
107	KEFLVVASV	Hematopoietic protein 1	Q52LW0
108	ITEPLPELQL	Heparan sulfate glucosamine 3-O-	Q8IZT8

		sulfotransferase 5	
109	KLRKEKEEP	Hepatocellular carcinoma-associated antigen 66	Q9NYH9
110	RLFVGSIPK	Heterogeneous nuclear ribonucleoprotein R (hnRNP R)	O43390
111	RMLPHAPGV	Histone deacetylase 1 (HD1)	Q13547
112	SPNMNAVISL	Histone deacetylase 9 (HD9) (HD7B) (HD7)	Q9UKV0
113	EFIDLLKKM	Homeodomain-interacting protein kinase 2 (EC 2.7.11.1)	Q9H2X6
114	SLLENLEKI	Heterogeneous nuclear ribonucleoprotein C-like 1	O60812
115	DQINIETKN	Regulator of nonsense transcripts 2	Q9HAU5
116	FNITYLDID	Interferon-inducible double stranded RNA-dependent protein kinase activator A	O75569
117	ILDLIDDAW	Anaphase promoting complex subunit 13	Q9BS18
118	QLAQFVHEV	Probable ATP-dependent RNA helicase DDX11	Q96FC9
119	VTYLEDYSA	Bcl-2-like 13 protein (Mil1 protein) (Bcl-rambo)	Q9BXK5
120	FLDDVVHSL	Jumonji domain-containing protein 1C	Q15652
121	TVMDEIHTV	Cell-cycle and apoptosis regulatory protein 1	Q6X935
122	KLISELQKL	Telomere-associated protein RIF1	Q5UIP0
123	KVIDEIYRV	F-box only protein 28	Q9NVF7
124	ELLENIIKN	Putative cell cycle control protein	Q9NXZ0
125	LADISLHDPV	ATP-dependent RNA helicase DDX31	Q9H8H2
126	FMMPQSLGV	Cysteine protease ATG4B	Q9Y4P1
127	FLTDYLNDL	BCoR protein (BCL-6 corepressor)	Q6W2J9
128	EFIPEPEK	Tubulin--tyrosine ligase-like protein 12	Q14166
129	FIFDVHVHEV	Plexin-B2 precursor (MM1)	O15031
130	ILEVTNNLE	Zinc finger and BTB domain-containing protein 5	O15062
131	ILSKKDLPL	Centrosome-associated protein 350	Q8WY20
132	KLLPYVGLLQ	Human homolog of Mus SLIT and NTRK-like protein 5	Q810B7
133	QLKSLIQID	Human homolog of Mus SLIT and NTRK-like protein 5	Q810B7
134	SLLNNPLSI	Nischarin	Q6PIB4
135	KLVEVIEEV	KIAA1598 protein	Q9HCH4
136	ILIDKSGKLEL	Bone specific CMF608	Q6WRI0
137	TVMDSKIVQV	Importin alpha-7 subunit (Karyopherin alpha-6)	O60693
138	VMDSKIVQV	Importin alpha-7 subunit (Karyopherin alpha-6)	O60693
139	YQDPLDPTRSV	InaD-like protein (Inadl protein) (hINADL)	Q8NI35
140	GLFPWTPKL	InaD-like protein (Inadl protein) (hINADL)	Q8NI35
141	NMYGKVVTV	Transcription elongation factor SPT5 (hSPT5)	O00267
142	QNVQVNQKV	Inositol-trisphosphate 3-kinase B	P27987
143	SLINQMTQV	Type I inositol-3,4-bisphosphate 4-phosphatase	Q96PE3
144	YYEKLHTYF	Integrin beta-4 precursor (GP150) (CD104 antigen)	P16144
145	SQQNTDNLV	Gap junction alpha-5 protein (Connexin-40)	P36382
146	WLDETLAQV	Kelch-like protein 8	Q9P2G9

147	ANYLDSMYI	ADAM 9 precursor	Q13443
148	HLWNSIHGL	Neighbor of BRCA1 gene 1 protein	Q14596
149	HLTYLNVYL	Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	Q92620
150	RTKLTDIQI	HUMAN CTCL tumor antigen HD-CL-04	Q548S1
151	RECKYDLPP	Importin-13 (Imp13) (Ran-binding protein 13)	O94829
152	QLTKIQTEL	KIAA0769 protein	O94868
153	TVNILIVDQN	Protocadherin-10 precursor	Q9P2E7
154	YLFDLPLKV	Leucine-rich repeats calponin homology domaincontaining2	Q5VUJ6
155	NLAKDNEVL	Ankyrin repeat domain 18B	Q5W0G2
156	SGDKLKLDQT	Kin17 protein (HsKin17 protein)	O60870
157	KLTDYQVTL	Kinesin-like protein KIF13A	Q9H1H9
158	KIQEILTQV	Putative RNA binding protein KOC	O00425
159	YLDEQIKKV	HUMAN Kinesin-like protein KIF13A	Q9H1H9
160	RLASYLDRV	Keratin, type I cytoskeletal 18 (Cytokeratin-18)	P05783
161	ALLNIKVKL	Keratin, type I cytoskeletal 18 (Cytokeratin-18)	P05783
162	KAITAPVSL	Lethal(3)malignant brain tumor-like protein	Q9Y468
163	LKAFKVAYS	Lactadherin precursor (Milk fat globule-EGF factor 8)	Q08431
164	RLAVYIDRV	Lamin-A/C (70 kDa lamin)	P02545
165	EMKVSDLDR	Laminin gamma-1 chain precursor (Laminin B2 chain)	P11047
166	PSTCPDGFKI	Mitogen-activated protein kinase kinase kinase 13	O43283
167	RKGIIDVNL	Leukemia virus receptor 2	Q08357
168	LLFDRPMHV	Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	P52272
169	FLSELTQQL	Macrophage migration inhibitory factor (MIF)	P14174
170	SLLSHVEQL	Mitotic spindle assembly checkpoint protein MAD2B	Q9UI95
171	KLILRLHKL	Mitogen-activated protein kinase kinase kinase 4	Q9Y6R4
172	RLTHHPVYI	Serine/threonine/tyrosine-interacting-like protein 1	Q9Y6J8
173	QDNLEKLLQ	Microtubule-associated serine/threonine-protein kinase 2	Q6P0Q8
174	FLFGEVHKA	MCM10 protein	Q7L590
175	KVIVLVNKVLL	Interferon-induced helicase C domain-containing protein 1	Q9BYX4
176	QILSLEEKI	Melanoma ubiquitous mutated protein	Q2TAK8
177	VLKEIVERV	Cytoplasmic activation/proliferation-associated protein 1	Q14444
178	SLLDEFYKL	Cytoplasmic activation/proliferation-associated protein 1	Q14444
179	TLNQNGYTLV	Met proto-oncogene (tyrosine kinase)	P08581
180	SIKDYEQAN	Mitotic kinesin-related protein	Q96Q89
181	VLISKELISL	(Mitotic spindle-associated protein p126)	Q96R06
182	LIEKVQEAR	Myeloid/lymphoid or mixed-lineage leukemia	Q9UMN6

		protein 4	
183	VLAETLTQV	MOZ/CBP protein	Q712H6
184	DTNADKQLS	Migration inhibitory factor-related protein 14	P06702
185	FVNDVNLEN	Multiple PDZ domain protein	O75970
186	SENKLILMK	RUFY2	Q8IW33
187	YLNDGLWHM	Multiple copies in a T-cell malignancies	Q9ULC4
188	GTTLRNLEI	DNA mismatch repair protein Msh3	P20585
189	SPPTLNGAPSP	Protein CBFA2T2 (MTG8-like protein) (MTG8-related protein 1)	O43439
190	LLAEKVEQL	Tumor suppressor candidate 3 (N33 protein)	Q13454
191	LANARGLGLQ	Nebulin-related anchoring protein	Q8TCH0
192	VNRIGQESLE	Neural cell adhesion molecule 1, 1	P13592
193	YLEIQGITR	Neurotrimin precursor	Q9P121
194	EALENNKEL	Ninein	Q8N4C6
195	HLLERVDQV	Ninein	Q8N4C6
196	PERTQLLYL	Notch homolog 2	Q5VTD0
197	NGGTCEDGIN	Neurogenic locus notch homolog protein 1 precursor	P46531
198	QSAADYLGAL	Neurogenic locus notch homolog protein 3 precursor	Q9UM47
199	ALLVVLSPPAL	Neurogenic locus notch homolog protein 4 precursor	Q99466
200	LRLDXLFKL	Plexin-A1 precursor (Semaphorin receptor NOV)	Q9UIW2
201	WLIEDGKVV	HUMAN NPD011	Q9H2R7
202	LLREKVEFL	Nuclear factor erythroid 2-related factor 1 (Transcription factor LCR-F1)	Q14494
203	TLFDYEVRL	Ubiquitin-like PHD and RING finger domain-containing protein 1	Q96T88
204	SILKVVINN	Nucleic acid helicase DDXx	Q8IWW2
205	KLAENIDAQL	Nucleoporin 62kDa (NUP62 protein)	Q6GTM2
206	SLLTDEEDVD	Nuclear pore complex protein Nup98-Nup96 precursor	P52948
207	QLEKKLME	Nucleoprotein TPR	P12270
208	GLDPLGYEIQ	Nuclear pore complex protein Nup107	P57740
209	ALLDRIVSV	Nuclear pore complex protein Nup205	Q92621
210	KILDLETQL	ODF2 protein	Q6PJQ8
211	VTWLKETEV	(5T4 oncofetal trophoblast glycoprotein	Q13641
212	FLDGHDLQL	(Megacaryocytic acute leukemia protein	Q969V6
213	KTTEVLDASA	Ovarian cancer related tumor marker CA125 -	Q8WXI7
214	TITNLQYGE	Ovarian cancer related tumor marker CA125 -	Q8WXI7
215	KIDALSSEKL	Centrosomal protein of 70 kDa (Cep70 protein)	Q8NHQ1
216	LDTPSQPVNN	Inhibitor of growth protein 3	Q9NXR8
217	VLDELKNMKC	P53 inducible protein	Q9UN29
218	NLLPKLHIV	Chloride intracellular channel protein 4	Q9Y696
219	SQGAVGLAGV	Protein patched homolog 1 (PTC1) (PTC)	Q13635

220	ELKKINYQV	Protein patched homolog 1 (PTC1) (PTC)	Q13635
221	GLLPQVNTPV	Pecanex-like protein 1 (Pecanex homolog) -	Q96RV3
222	KAYDVEREL	GC-1-related estrogen receptor alpha coactivator short isoform	Q8TDE4
223	DVLESWLDF	PHD finger	Q86U89
224	FAYLLTYMA	Transmembrane protein 115 (Protein PL6)	Q12893
225	GLIDSLVHYV	Plakophilin-2	Q99959
226	REDHPARP	Plectin 6	Q6S380
227	FLLDPVKGERL	Plectin 1 (PLTN) (PCN) (Hemidesmosomal protein 1) (HD1)	Q15149
228	RGQNLDVVQ	Plexin B1; plexin 5; semaphorin receptor	O43157
229	SLTGHISTV	Pleiotropic regulator 1	O43660
230	EPLRVPPDL	Blood vessel epicardial substance (hBVES)	Q8NE79
231	SLLQHLIGL	Melanoma antigen preferentially expressed in tumors	P78395
232	ILMGVLKEV	Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	O43143
233	VLFENTDSVHL	HUMAN RNA-binding protein 34	P42696
234	KTDKTLVLL	Profilin-1	P07737
235	GLIEILKKV	Programmed cell death protein 5 (TFAR19 protein)	O14737
236	SLADIAQKL	26S proteasome non-ATPase regulatory subunit 3	O43242
237	QLVDIIEKV	Proteasome activator complex subunit 3 (PA28gamma)	P61289
238	SLLKVDQEV	Proteasome activator complex subunit 3 (PA28gamma)	P61289
239	SLLPPDALVGL	Protein transport protein Sec23B	Q15437
240	KLFNDAIRL	Rab-like protein 2B	Q9UNT1
241	FENQEVQAI	Cell cycle checkpoint protein RAD17	O75943
242	EYVEKFYRI	DNA repair protein RAD50 (EC 3.6.-.-) (hRAD50)	Q92878
243	QIDEIRDK	DNA repair protein RAD50 (EC 3.6.-.-) (hRAD50)	Q92878
244	FLHEKLESL	Ras GTPase-activating protein 1	P20936
245	VMFNGKVYL	Receptor-interacting factor 1	Q86XS4
246	DVLFALFSKL	Retinoblastoma-associated protein (PP110) (P105-RB)	P06400
247	GLNEEIARV	Retinoblastoma-associated protein HEC	O14777
248	FLFQEPRSI	Retinoblastoma-associated protein RAP140	Q9UK61
249	KEVDILNLP	Retinoblastoma-binding protein 1	P29374
250	TMVDRIEEV	Jumonji/ARID domain-containing protein 1A	P29375
251	DVYEDELVP	RNA-binding protein	Q8NI52
252	VMLGGRNIKV	Ro ribonucleoprotein-binding protein 1	Q9UHX1
253	FLLEPQMKV	Secreted and transmembrane protein 1 precursor	Q8WVN6
254	SMNRGGYMP	Semaphorin-6D precursor	Q8NFY4
255	TLSERLWLG	Shb-like adapter protein, Shf	Q7M4L6

256	VLWDRTFSL	Signal transducer and activator of transcription 1-alpha/beta	P42224
257	NVNPFTKPP	Signal transducer and activator of transcription 3	P40763
258	QTDVDNDLV	Thrombospondin-2 precursor	P35442
259	KMDDPDYWRTV	Ribosome biogenesis protein BOP1 (Block of proliferation 1 protein)	Q14137
260	LQRRKPTGAF	FRAS1-related extracellular matrix protein 2 precursor	Q5SZK8
261	ILDKKVEKV	Heat shock protein HSP 90-beta (HSP 84) (HSP 90)	P08238
262	KLSAEVESLK	Sarcoma antigen NY-SAR-41 (NY-SAR-41)	Q5T9S5
263	RVLPYPFTH	U3 small nucleolar RNAassociated protein 14 homolog A	Q9BVJ6
264	QDNIKELEL	Chromosome-associated kinesin KIF4A (Chromokinesin)	O95239
265	ILKQRDNEI	Kinesin-like protein KIF6	Q6ZMV9
266	QNELDNVSTL	Myosin-10 (Myosin heavy chain, nonmuscle IIb)	P35580
267	SYVNLPTIAL	34/67 kDa laminin receptor) (Colon carcinoma laminin-binding protein) (NEM/1CHD4) (Multidrug resistance- associated protein MGr1-Ag)	P08865
268	SLADLQNDEV	40S ribosomal protein S3a	P61247
269	YLLDLHSYL	TEB4 protein	O14670
270	TLAEVSTRL	Serine/threonine-protein kinase SNF1-like kinase 1	P57059
271	RLWEEAVKA	Zinc finger protein 161 (Putative transcription factor DB1)	Q14119
272	SLKTLMLR	Slit homolog 2 protein precursor (Slit-2)	O94813
273	EIKKKFKL	FYN-binding protein (FYN-T-binding protein)	O15117
274	VHKEMFIMV	Jumonji/ARID domain-containing protein 1C	P41229
275	RVADRLYGV	Sorting nexin-4	O95219
276	TLDENHPSI	Spermatogenesis-associated protein 7 (HSD3)	Q9P0W8
277	TLAEIAKVEL	Non-POU domain-containing octamer-binding protein	Q15233
278	DVAVEAIRL	Cohesin subunit SA-1 (Stromal antigen 1)	Q8WVM7
279	SINPKRAKL	Suppressor of hairy wing homolog 2 (5'OY11.1)	Q86YH2
280	SLFATEQL	Synaptogyrin-3	O43761
281	ALNELLQHV	Talin-1	Q9Y490
282	SNFGNEKL	TRA@ protein	Q6PIP7
283	FLLDKKIGV	T-complex protein 1 subunit beta (TCP-1-beta)	P78371
284	RSLAASNPIL	Telomerase-binding protein EST1A	Q86US8
285	EMESLTGHQ	Tumor endothelial marker 6 (Hypothetical protein TEM6)	Q96PE0
286	LDFQEELEV	Ras GTPase-activating-like protein IQGAP2	Q13576
287	ILYELQVEL	TMC4 protein	Q7Z5M3
288	TNIEDGVFET	Toll-like receptor 8 precursor	Q9NR97
289	IAAKILSYN	DNA topoisomerase I, mitochondrial precursor	Q969P6
290	LYGRHFNYL	Topoisomerase-related function protein 4-2	Q8NDF8

291	NLFNKYPAL	Plastin-3 (T-plastin)	P13797
292	YLDEIVKEV	To activated MET oncogene	Q5SWY0
293	RTHMLSSL	Transcript Y 5	Q9BXH6
294	LNVDTPFPL	Transducer of regulated CREB protein 3	Q6UUV7
295	ILYELKVEL	Transmembrane channel-like protein 4	Q7Z404
296	VFPTHVFPT	Tubulin, gamma complex associated protein 3	Q5T9Y2
297	KELAELRESTS	Tumor necrosis factor ligand superfamily member 6	P48023
298	LTDKEGWIL	Tumor necrosis factor, endothelial (B12 protein)	Q13829
299	VVTYKNENI	Netrin receptor DCC precursor	P43146
300	NEKIKKDEL	U1 small nuclear ribonucleoprotein A	P09012
301	ILDESHERV	U6 snRNA-associated Sm-like protein LSm8	O95777
302	NLYSDYILN	Ubiquitin-protein ligase E3A	Q05086
303	RYVNGHAK	Ubiquitin carboxyl-terminal hydrolase 3	Q9Y6I4
304	YLYDLNHTL	UNC93 homolog B1 (UNC-93B protein) (hUNC93B1)	Q9H1C4
305	KLGSVPVTV	CCDC73 protein	Q6P5Q7
306	ALWERIEGV	Caspase recruitment domain-containing protein 10	Q9BWT7
307	ILEKKVEKV	Heat shock protein HSP 90-alpha (HSP 86)	P07900
308	KASQLGIFISKV	PDZ domain-containing protein 11	Q5EBL8
309	KILEVMHTK	Dedicator of cytokinesis 11	Q5JSL3
310	LLAEEARKL	Laminin gamma-1 chain precursor (Laminin B2 chain)	P11047
311	LLGICFCIA	Multidrug resistance-associated protein 8	Q96J66
312	MISLPGPLVTN	Endothelial cell-selective adhesion molecule precursor	Q96AP7
313	NVMNLIDFV	Voltage-gated potassium channel KCNA7	Q96RP8
314	SLLDKIIGA	Polymerase I and transcript release factor	Q6NZI2
315	TLDEKIEKV	Probable ATP-dependent RNA helicase DDX	Q96GQ7
316	FYDIETLK	Vascular endothelial growth factor D (VEGF-D)	O43915
317	WMAPESIFDKI	Vascular endothelial growth factor receptor 1 precursor	P17948
318	LLDQQNPDE	Proto-oncogene C-crk (P38) (Adapter molecule crk)	P46108
319	VMFKKIKSFEV	VDUP1 protein (Thioredoxin interacting protein)	Q9H3M7
320	KLLEGEESRISL	Vimentin	P08670
321	RILGAVAKV	Vinculin (Metavinculin)	P18206
322	SLSMVNHRL	Integrin alpha-3 precursor (VLA-3 alpha chain)	P26006
323	SLTDKVQEA	Myeloid/lymphoid or mixed-lineage leukemia	Q59PF2
324	KMDDPDYWRTV	Ribosome biogenesis protein BOP1	Q14137
325	KLDPTKTTL	NDRG1 protein	Q92597
326	HLTYLNVYL	Pre-mRNA splicing factor ATP-dependent RNA helicase PRP16	Q92620
327	ALWDKLFNL	Nesprin 2 (Nuclear envelope spectrin repeat protein 2)	Q9NU50
328	KIMDQVQQA	Adenomatous polyposis coli	P25054

329	RLQEDPPAGV	Ubiquitin conjugating enzyme E2	P49459
330	KLDVGNAEV	B cell receptor-associated protein BAP31 (CDM protein)	P5572
331	FLYDDNQRV	Topoisomerase II-alpha	P11388
332	FLYDDNQRV	Topoisomerase II beta	Q02880
333	ALMEQQHYV	Integrin beta8 subunit precursor	P26012
334	YLMDTSGKV	Replication Protein A	P27694
335	ILDDIGHGV	Abl Binding protein 3	U31089
336	LLDRPLATV	Cyclin I	Q14094
337	LLIDDKGTIKL	Cell Division Control Protein 2 (CDC2)	P06493
338	RLYPWGVVEV	Septin 2 (NEDD5)	Q15019
339	KLQELNYNL	STAT1 alpha/beta	P42224
340	ILIEHLYGL	LDL Receptor-related protein (LRP)	Q07954
341	YLIELIDRV	TACE (ADAM17)	NP-068604
342	NLMEQPIKV	Junction plakoglobin (gamma catenin)	P14923
343	FLAEDALNTV	EDDR1 (rtk6), h-RYK	Q08345
344	TLLNVIKSV	IP3 receptor type II	Q14571
345	MLKDIIKEY	Melanoma-associated antigen D2 (MAGE-D2 antigen)	Q9UNF1
346	TSYVKVLEH	Melanoma-associated antigen 4 (MAGE-4 antigen)	P43358
347	HEYLKAFKV	HUMAN Retinoblastoma-like protein 2	Q08999
348	VLMTEDIKL	Eukaryotic translation initiation factor 4 gamma 1	Q04637
349	AVDEDRKMYL	Cullin-2 (CUL-2)	Q13617
350	SLFEKGLKNV	F-box/LRR-repeat protein 5	Q9UKA1
351	LLSNNNQAL	Ras-GTPase-activating protein-binding protein 1	Q13283
352	LLSNNNQAL	Ras-GTPase-activating protein-binding protein 1	Q13283
353	FLADPDTVNHLL	Sorting nexin 14, isoform a	Q6NUI7
354	ILMEHIHKL	60S ribosomal protein L19	P84098

Table 3. Parent Protein Identification and SwissProt Identification Number for full-length sequences 355-693.

Parent Protein Identification	SwissProt Id No.
BCL-6 corepressor long isoform	Q6W2J9
Heat shock protein HSP 90-beta (HSP 84)	P08238
2'-5'oligoadenylate synthetase 3	Q2HJ14
26S protease regulatory subunit 4 (P26s4)	P62191
26S proteasome non-ATPase regulatory subunit 7	P51665
40S ribosomal protein S16	P62249
40S ribosomal protein S6 (Phosphoprotein NP33)	P62753
40S ribosomal protein S9	P46781
60S ribosomal protein L10a (CSA-19)	P62906
A kinase anchor protein 10, mitochondrial precursor	O43572
Activated T-cell marker CD109	Q6YHK3
Activin receptor type 2A precursor (EC 2.7.11.30)	P27037
ADAM19 protein	Q8TBU7
AP-1 complex subunit beta-1	Q10567
Lung alpha/beta hydrolase protein 1	Q96SE0
Alpha-actinin-3	Q08043
Ankyrin repeat and SOCS box protein 17	Q8WXJ9
Anti-colorectal carcinoma heavy chain	Q65ZQ1
APOBEC1 complementation factor	Q9NQ94
Probable DNA dC->dU-editing enzyme APOBEC-3D	Q96AK3
Apolipoprotein-L4 precursor (Apolipoprotein L-IV)	Q9BPW4
Apoptosis stimulating of p53 protein 1	Q96KQ4
Nucleoporin 188kDa (arachin)	Q5SRE5
Protein ariadne-1 homolog (ARI-1)	Q9Y4X5
Set1/Ash2 histone methyltransferase complex subunit ASH2	Q9UBL3
Ubiquitin carboxyl-terminal hydrolase 20	Q9Y2K6
Bcl-2 related ovarian killer	Q9UL32
Cell growth inhibiting protein 39	Q2TTR2
BH3-interacting domain death agonist (BID)	P55957
Bone morphogenetic protein receptor type-2 precursor	Q13873
BRCA1 associated RING domain 1 variant	Q53F80
Breast cancer type 2 susceptibility protein	P51587
BRCA1/BRCA2-containing complex subunit 45	Q9NXR7
Breast cancer 1 early onset	Q3LRJ0
Breast and ovarian cancer susceptibility protein	Q7KYU6
BTG2 protein (NGF-inducible anti-proliferative protein PC3)	P78543
Nuclear protein 5qNCA	Q7LBC6
Integrin alpha-3 precursor (Galactoprotein B3)	P26006

Hepatocellular carcinoma- associated antigen 520	O43745
Calpain-11 (EC 3.4.22.-)	Q9UMQ6
Alpha-1 catenin (Cadherin-associated protein) (Alpha E-catenin)	P35221
Neural cell adhesion molecule variant	Q59FY0
Ribosomal L1 domain-containing protein 1	O76021
CENP-F kinetochore protein (Centromere protein F)	P49454
Pericentriol material 1	Q15154
Cervical cancer suppressor gene 5	Q8NFX8
Vacuolar protein sorting 13A	Q96RL7
Chromodomain-helicase-DNA-binding protein 2	O14647
Adiponutrin (iPLA2-epsilon)	Q9NST1
CUB and sushi domain-containing protein 3 precursor	Q7Z407
Cullin-7 (CUL-7)	Q14999
Cyclic AMP-dependent transcription factor ATF-4	P18848
Cyclin A/CDK2-associated protein p19	P63208
Cyclin-A1	P78396
Cyclin M3, isoform 1	Q8NE01
CPEB2 protein	Q3B8N6
Probable ATP-dependent RNA helicase DDX5	P17844
Dedicator of cytokinesis protein 1	Q14185
Development and differentiation-enhancing factor 2	O43150
G2 and S phase expressed protein 1	Q9NYZ3
ATP-dependent helicase PRIC285	Q9BYK8
Transcription elongation factor SPT5 (DLC-1)(deleted in liver cancer-1)	O00267
DNA damage-binding protein 1	Q16531
DNA excision repair protein ERCC-6	Q03468
DNA polymerase alpha subunit B	Q14181
DNA replication licensing factor MCM2	P49736
DNA2-like homolog	P51530
Estrogen response element binding protein	O77798
DNA damage-inducible transcript 3 (	P35638
DNA-directed RNA polymerase I largest subunit	O95602
DRE1 protein	Q9NXT9
Dynactin-1 (150 kDa dynein-associated polypeptide)	Q14203
Dynein heavy chain, cytosolic (DYHC)	Q14204
Echinoderm microtubule associated protein-like 5	Q6UYC9
ECT2 protein	Q9H8V3
Endothelial differentiation-related factor 1 (EDF-1)	O60869
Developmentally-regulated endothelial cell locus 1 protein)	O43854
Enhancer of filamentation 1 (HEF1)	Q14511
Band 4.1-like protein 3 (4.1B)	Q9Y2J2
Epidermal growth factor receptor substrate 15	P42566
Epithelial membrane protein 3 (EMP-3) (YMP protein)	P54852

Eukaryotic translation initiation factor 4 gamma 1	Q04637
Protocadherin Fat 2 precursor (hFat2)	Q9NYQ8
Fibroblast growth factor receptor-like 1 precursor	Q8N441
Fibroblast growth factor receptor 4 precursor	P22455
Fibroblast growth factor receptor 2 precursor	P21802
FKSG73	Q9BWW1
Protein flightless-1 homolog	Q13045
Filamin-A (Alpha-filamin) (Filamin-1)	P21333
Flotillin-2 (Epidermal surface antigen) (ESA)	Q14254
Serine/threonine-protein kinase ATR	Q13535
Frizzled 5 precursor (Frizzled-5)	Q13467
G1 to S phase transition protein 1 homolog	P15170
Golgin subfamily B member 1 (Giantin)	Q14789
Growth factor receptor-bound protein 14	Q14449
CTCL tumor antigen se1-1	Q8IWJ2
GTP-binding protein Rit1	Q92963
Heat shock protein 75 kDa, mitochondrial precursor (HSP 75) (Tumor necrosis factor type 1 receptor-associated protein)	Q12931
Tumor rejection antigen (Gp96)	Q5CAQ5
Heat-shock protein beta-3 (HspB3)	Q12988
Low-density lipoprotein receptor-related protein 5 precursor	Q75197
Hematopoietic protein 1	Q52LW0
Heparan sulfate glucosamine 3-O-sulfotransferase 5	Q8IZT8
Hepatocellular carcinoma-associated antigen 66	Q9NYH9
Heterogeneous nuclear ribonucleoprotein R (hnRNP R)	O43390
Histone deacetylase 1 (HD1)	Q13547
Histone deacetylase 9 (HD9) (HD7B) (HD7)	Q9UKV0
Homeodomain-interacting protein kinase 2	Q9H2X6
Cullin-2 (CUL-2)	Q13617
Heterogeneous nuclear ribonucleoprotein C-like 1	O60812
Regulator of nonsense transcripts 2	Q9HAU5
Interferon-inducible double stranded RNA-dependent protein kinase activator A	O75569
Anaphase promoting complex subunit 13	Q9BS18
Probable ATP-dependent RNA helicase DDX11	Q96FC9
Bcl-2-like 13 protein (Mil1 protein) (Bcl-rambo)	Q9BXK5
Jumonji domain-containing protein 1C	Q15652
Cell-cycle and apoptosis regulatory protein 1	Q6X935
Telomere-associated protein RIF1	Q5UIP0
F-box only protein 28	Q9NVF7
Putative cell cycle control protein (DEP domain containing 1)	Q9NXZ0
ATP-dependent RNA helicase DDX31	Q9H8H2
Cysteine protease ATG4B	Q9Y4P1
BCoR protein (BCL-6 corepressor)	Q6W2J9

Tubulin--tyrosine ligase-like protein 12	Q14166
Plexin-B2 precursor (MM1)	O15031
Zinc finger and BTB domain-containing protein 5	O15062
Centrosome-associated protein 350	Q8WY20
Human homolog of Mus SLIT and NTRK-like protein 5 precursor	Q810B7
Nischarin	Q6PIB4
KIAA1598 protein	Q9HCH4
Bone specific CMF008	Q6WRI0
Importin alpha-7 subunit (Karyopherin alpha-6)	O00693
ImaD-like protein (Inadl protein)	Q8NI35
Transcription elongation factor SPT5 (hSPT5)	O00267
Inositol-trisphosphate 3-kinase B	P27987
Type I inositol-3,4-bisphosphate 4-phosphatase	Q96PE3
Integrin beta-4 precursor (GP150) (CD104 antigen)	P16144
Gap junction alpha-5 protein (Connexin-40)	P36382
Kelch-like protein 8	Q9P2G9
ADAM 9 precursor	Q13443
Neighbor of BRCA1 gene 1 protein	Q14596
Pre-mRNA-splicing factor ATP-dependent RNA helicase PRP16	Q92620
HUMAN CTCL tumor antigen HD-CL-04	Q548S1
Importin-13 (Imp13) (Ran-binding protein 13)	O94829
KIAA0769 protein	O94868
Protocadherin-10 precursor	Q9P2E7
Leucine-rich repeats and calponin homology (CH) domain containing 2	Q5VUJ6
Ankyrin repeat domain 18B	Q5W0G2
Kin17 protein (HsKin17 protein)	O60870
Kinesin-like protein KIF13A (Kinesin-like protein RBKIN)	Q9H1H9
Putative RNA binding protein KOC	O00425
Keratin, type I cytoskeletal 18 (Cytokeratin-18)	P05783
Lethal(3)malignant brain tumor-like protein (L(3)mbt-like)	Q9Y468
Lactadherin precursor (Milk fat globule-EGF factor 8) (	Q08431
Lamin-A/C (70 kDa lamin)	P02545
Laminin gamma-1 chain precursor (Laminin B2 chain)	P11047
Mitogen-activated protein kinase kinase kinase 13 (EC 2.7.11.25)	O43283
Leukemia virus receptor 2	Q08357
Heterogeneous nuclear ribonucleoprotein M (hnRNP M)	P52272
Macrophage migration inhibitory factor (MIF)	P14174
Mitotic spindle assembly checkpoint protein MAD2B	Q9UI95
Mitogen-activated protein kinase kinase kinase 4	Q9Y6R4
Serine/threonine/tyrosine-interacting-like protein 1	Q9Y6J8
Microtubule-associated serine/threonine-protein kinase 2	Q6P0Q8

MCM10 protein	Q7L590
Interferon-induced helicase C domain-containing protein 1	Q9BYX4
Melanoma ubiquitous mutated protein	Q2TAK8
GPI-anchored protein p137 (p137GPI)	Q14444
Hepatocyte growth factor receptor precursor	P08581
Mitotic kinesin-related protein	Q96Q89
Mitotic spindle-associated protein p126	Q96R06
Myeloid/lymphoid or mixed-lineage leukemia protein 4	Q9UMN6
MOZ/CBP protein	Q712H6
Calgranulin B (Migration inhibitory factor-related protein 14)	P06702
Multiple PDZ domain protein (Multi PDZ domain protein 1)	O75970
RUFY2 (Run and FYVE domain-containing protein Rabip4	Q8IW33
Multiple copies in a T-cell malignancies	Q9ULC4
DNA mismatch repair protein Msh3	P20585
Protein CBFA2T2 (MTG8-like protein)	O43439
Tumor suppressor candidate 3 (N33 protein)	Q13454
Nebulin-related anchoring protein	Q8TCH0
Neural cell adhesion molecule 1, 1	P13592
Neurotrimin precursor	Q9P121
Ninein	Q8N4C6
Notch homolog 2	Q5VTD0
Neurogenic locus notch homolog protein 1 precursor	P46531
Neurogenic locus notch homolog protein 3 precursor	Q9UM47
Neurogenic locus notch homolog protein 4 precursor	Q99466
Plexin-A1 precursor (Semaphorin receptor NOV)	Q9UIW2
HUMAN NPD011	Q9H2R7
Nuclear factor erythroid 2-related factor 1	Q14494
Ubiquitin-like PHD and RING finger domain-containing protein 1	Q96T88
Nucleic acid helicase DDXx	Q8IWW2
Nucleoporin 62kDa (NUP62 protein)	Q6GTM2
Nuclear pore complex protein Nup98-Nup96 precursor	P52948
Nucleoprotein TPR	P12270
Nuclear pore complex protein Nup107	P57740
Nuclear pore complex protein Nup205	Q92621
ODF2 protein	Q6PJQ8
Trophoblast glycoprotein precursor	Q13641
Megacaryocytic acute leukemia protein	Q969V6
Ovarian cancer related tumor marker CA125 -	Q8WXI7
Centrosomal protein of 70 kDa (Cep70 protein) (p10-binding protein)	Q8NHQ1
F-box/LRR-repeat protein 5 (F-box and leucine-rich repeat protein 5) (F-box protein FBL4/FBL5)	Q9UKA1

Inhibitor of growth protein 3	Q9NXR8
P53 inducible protein	Q9UN29
Chloride intracellular channel protein 4	Q9Y696
Protein patched homolog 1 (PTC1)	Q13635
Pecanex-like protein 1 (Pecanex homolog) -	Q96RV3
GC-1-related estrogen receptor alpha coactivator short isoform	Q8TDE4
PHD finger	Q86U89
Transmembrane protein 115 (Protein PL6)	Q12893
Plakophilin-2	Q99959
Plectin 6	Q6S380
Plectin 1 (PLTN) (PCN) (Hemidesmosomal protein 1) (HD1)	Q15149
Plexin B1; plexin 5; semaphorin receptor	O43157
Pleiotropic regulator 1	O43660
Blood vessel epicardial substance (hBVES)	Q8NE79
Melanoma antigen preferentially expressed in tumors	P78395
Putative pre-mRNA-splicing factor ATP-dependent RNA helicase DHX15	O43143
HUMAN RNA-binding protein 34 (RNA-binding motif protein 34)	P42696
Prolyl 4-hydroxylase alpha-1 subunit precursor (EC 1.14.11.2) (4-PH alpha-1) (Procollagen-proline,2-oxoglutarate-4-dioxygenase alpha-1 subunit)	P13674
Profilin-1	P07737
Programmed cell death protein 5 (TFAR19 protein)	O14737
26S proteasome non-ATPase regulatory subunit 3	O43242
Proteasome activator complex subunit 3	P61289
Protein transport protein Sec23B	Q15437
Rab-like protein 2B	Q9UNT1
Cell cycle checkpoint protein RAD17 (hRad17)	O75943
DNA repair protein RAD50 (EC 3.6.-.-) (hRAD50)	Q92878
Ras GTPase-activating protein 1 (GTPase-activating protein)	P20936
Ras-GTPase-activating protein-binding protein 1 (EC 3.6.1.-) (ATP- dependent DNA helicase VIII) (GAP SH3-domain-binding protein 1) (G3BP- 1) (HDH-VIII)	Q13283
Receptor-interacting factor 1	Q86XS4
Retinoblastoma-associated protein (PP110) (P105-RB)	P06400
Retinoblastoma-associated protein HEC (Kinetochore associated 2)	O14777
Retinoblastoma-associated protein RAP140	Q9UK61
AT-rich interactive domain-containing protein 4A (	P29374
Jumonji/ARID domain-containing protein 1A	P29375
RNA-binding protein	Q8NI52
Ro ribonucleoprotein-binding protein 1 (SIAHBP1 protein)	Q9UHX1
Secreted and transmembrane protein 1 precursor (Protein	Q8WVN6

K12)	
Semaphorin-6D precursor	Q8NFY4
Serine/threonine/tyrosine-interacting protein (Protein tyrosine phosphatase-like protein)	Q8WUJ0
Shb-like adapter protein, Shf	Q7M4L6
Signal transducer and activator of transcription 1-alpha/beta	P42224
Signal transducer and activator of transcription 3	P40763
Thrombospondin-2 precursor	P35442
Ribosome biogenesis protein BOP1 (Block of proliferation 1 protein)	Q14137
LRRC58 protein	Q96CX6
FRAS1-related extracellular matrix protein 2 precursor (ECM3 homolog)	Q5SZK8
Eukaryotic translation initiation factor 3 subunit 8 (eIF3 p110) (eIF3c)	Q99613
Heat shock protein HSP 90-beta (HSP 84) (HSP 90)	P08238
Sarcoma antigen NY-SAR-41 (NY-SAR-41)	Q5T9S5
U3 small nucleolar RNA-associated protein 14 homolog A	Q9BVJ6
Chromosome-associated kinesin KIF4A (Chromokinesin)	O95239
Kinesin-like protein KIF6	Q6ZMV9
Myosin-10 (Myosin heavy chain, nonmuscle IIb)	P35580
Colon carcinoma laminin-binding protein) (NEM/1CHD4	P08865
40S ribosomal protein S3a	P61247
TEB4 protein	O14670
Serine/threonine-protein kinase SNF1-like kinase 1 (EC 2.7.11.1)	P57059
Zinc finger protein 161 (Putative transcription factor DB1)	Q14119
Slit homolog 2 protein precursor (Slit-2)	O94813
FYN-binding protein (FYN-T-binding protein)	O15117
Jumonji/ARID domain-containing protein 1C (SmcX protein)	P41229
Sorting nexin 14, isoform a	Q6NUI7
Sorting nexin-4	O95219
Spermatogenesis-associated protein 7	Q9P0W8
Non-POU domain-containing octamer-binding protein	Q15233
Cohesin subunit SA-1 (Stromal antigen 1) (SCC3 homolog 1)	Q8WVM7
Suppressor of hairy wing homolog 2 (5'OY11.1) (Zinc finger protein 632)	Q86YH2
Synaptogyrin-3	O43761
Talin-1	Q9Y490
TRA@ protein	Q6PIP7
T-complex protein 1 subunit beta (TCP-1-beta) (CCT-beta)	P78371

Telomerase-binding protein EST1A (Ever shorter telomeres 1A)	Q86US8
Tumor endothelial marker 6 (Hypothetical protein TEM6)	Q96PE0
Ras GTPase-activating-like protein IQGAP2	Q13576
TMC4 protein	Q7Z5M3
Toll-like receptor 8 precursor	Q9NR97
DNA topoisomerase I, mitochondrial precursor	Q969P6
Topoisomerase-related function protein 4-2	Q8NDF8
Plastin-3 (T-plastin)	P13797
Translocated promoter region (To activated MET oncogene)	Q5SWY0
Transcript Y 5	Q9BXH6
Transducer of regulated CREB protein 3	Q6UUV7
Transmembrane channel-like protein 4	Q7Z404
Tubulin, gamma complex associated protein 3	Q5T9Y2
Tumor necrosis factor ligand superfamily member 6	P48023
Tumor necrosis factor, alpha-induced protein 1, endothelial (B12 protein)	Q13829
Netrin receptor DCC precursor	P43146
U1 small nuclear ribonucleoprotein A	P09012
U6 snRNA-associated Sm-like protein LSm8	O95777
Ubiquitin-protein ligase E3A	Q05086
Ubiquitin carboxyl-terminal hydrolase 3	Q9Y6I4
UNC93 homolog B1 (UNC-93B protein) (hUNC93B1)	Q9H1C4
CCDC73 protein	Q6P5Q7
Caspase recruitment domain-containing protein 10	Q9BWT7
Heat shock protein HSP 90-alpha (HSP 86)	P07900
60S ribosomal protein L19	P84098
PDZ domain-containing protein 11	Q5EBL8
Dedicator of cytokinesis 11	Q5JSL3
Laminin gamma-1 chain precursor (Laminin B2 chain)	P11047
ATP-binding cassette transporter sub-family C member 11	Q96J66
Endothelial cell-selective adhesion molecule precursor	Q96AP7
Voltage-gated potassium channel KCNA7	Q96RP8
Polymerase I and transcript release factor (PTRF protein)	Q6NZI2
Probable ATP-dependent RNA helicase DDX27	Q96GQ7
Vascular endothelial growth factor D precursor (VEGF-D)	O43915
Vascular endothelial growth factor receptor 1 precursor	P17948
Proto-oncogene C-crk (P38) (Adapter molecule crk)	P46108
VDUP1 protein (Thioredoxin interacting protein)	Q9H3M7
Vimentin	P08670
Vinculin (Metavinculin)	P18206
Integrin alpha-3 precursor (Galactoprotein B3) (GAPB3) (VLA-3 alpha chain) (FRP-2) (CD49c antigen)	P26006

[Contains: Integrin alpha-3 heavy chain; Integrin alpha-3 light chain]	
Proto-oncogene protein Wnt-3 precursor	P56703
Myeloid/lymphoid or mixed-lineage leukemia	Q59FF2
Ribosome biogenesis protein BOP1 (Block of proliferation 1 protein)	Q14137
NDRG1 protein (N-myc downstream regulated gene 1 protein)	Q92597
Pre-mRNA splicing factor ATP-dependent RNA helicase PRP16	Q92620
Nesprin 2 (Nuclear envelope spectrin repeat protein 2)	Q9NU50
Adenomatous polyposis coli	P25054
Ubiquitin conjugating enzyme E2	P49459
B cell receptor-associated protein BAP31 (CDM protein) 6c6-AG	P5572
Topoisomerase II-alpha	P11388
Topoisomerase II beta	Q02880
Integrin beta8 subunit precursor	P26012
Replication Protein A	P27694
Abl Binding protein 3	U31089
Cyclin I	Q14094
Cell Division Control Protein 2 (CDC2)	P06493
Septin 2 (NEDD5)	Q15019
STAT1 alpha/beta	P42224
LDL Receptor-related protein (LRP)	Q07954
TACE (ADAM17)	NP-068604
Junction plakoglobin (gamma catenin)	P14923
EDDR1 (rtk6), h-RYK	Q08345
IP3 receptor type II	Q14571
Melanoma-associated antigen D2 (MAGE-D2 antigen)	Q9UNF1
Melanoma-associated antigen 4 (MAGE-4 antigen)	P43358
HUMAN Retinoblastoma-like protein 2	Q08999
Eukaryotic translation initiation factor 4 gamma 1	Q04637
Cullin-2 (CUL-2)	Q13617
F-box/LRR-repeat protein 5	Q9UKA1
Ras-GTPase-activating protein-binding protein 1	Q13283
Ras-GTPase-activating protein-binding protein 1	Q13283
Sorting nexin 14, isoform a	Q6NUI7
60S ribosomal protein L19	P84098

We claim:

1. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing any class I MHC molecule, comprising administering to said subject a composition comprising

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 in an amount sufficient to induce a CTL response to said tumor cells; or

at least one polypeptide comprising an epitopic peptide having at least one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 in an amount sufficient to induce a CTL response to said tumor cells.

2. The method of claim 1, wherein said amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 is the result of a conservative amino acid substitution.

3. The method of claim 1, wherein said amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 is the result of a substitution of one hydrophobic amino acid with another hydrophobic amino acid.

4. The method of claim 1, wherein said amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 is the result of an addition or deletion of one amino acid to or from said epitopic peptide.

5. The method of claim 1, wherein said composition further comprises an adjuvant.

6. The method of claim 5, wherein said adjuvant is selected from the group consisting of complete Freund's adjuvant, incomplete Freund's adjuvant, Montanide ISA-51, LAG-3, aluminum phosphate, aluminum hydroxide, alum, and saponin.

7. The method of claim 1, wherein said composition further comprises a cytokine.

8. The method of claim 5, wherein said cytokine is selected from the group consisting of IL-1, IL-2, IL-7, IL-12, IL-15, TNF, SCF and GM-CSF.

9. The method of claim 1, where in said composition further comprises a vehicle.

10. The method of claim 9, where said vehicle is selected from the group consisting of a liposome, an immunostimulating complex (ISCOM), and slow-releasing particles.

11. The method of claim 10, where in said liposome comprises an emulsion, a foam, a micel, an insoluble monolayer, a liquid crystal, a phospholipid dispersion, or a lamellar layer.

12. The method of claim 1, wherein said polypeptide consists of
an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354; or
an amino acid sequence having at least one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354.

13. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing any class I MHC molecule, said method comprising administering to said subject a composition comprising a polynucleotide comprising a nucleic acid sequence encoding
at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 in an amount sufficient to induce a CTL response to said tumor cells; or
at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group

consisting of SEQ ID NO: 1 to 354 in an amount sufficient to induce a CTL response to said tumor cells.

14. The method of claim 13, wherein said polynucleotide further comprises an expression vector.

15. The method of claim 14, wherein said expression vector is a plasmid or a nonreplicative viral vector.

16. The method of claim 14, wherein said expression vector is an RNA virus.

17. The method of claim 14, wherein said expression vector is a DNA virus.

18. The method of claim 15, wherein said nonreplicative viral vector is selected from the group consisting of vaccinia, fowlpox, Venezuelan equine encephalitis virus, and adenovirus.

19. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing HLA-A1, A11 or A2, said method comprising

administering to said subject induced CTLs in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines, said CTLs induced by a process comprising

inducing a cytotoxic T lymphocyte (CTL) *in vitro* that is specific for said tumor cells by contacting a precursor CTL with:

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 under conditions that generate a CTL response to said tumor cells; or

at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 under

conditions that generate a CTL response to said tumor cells.

20. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing any class I MHC molecule, said method comprising

administering to said subject induced CTLs in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines, said CTLs induced by a process comprising

inducing a cytotoxic T lymphocyte (CTL) *in vitro* that is specific for said tumor cells by contacting a precursor CTL with:

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 355 to 693 under conditions that generate a CTL response to said tumor cells; or

at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 355 to 693 under conditions that generate a CTL response to said tumor cells.

21. A method for inducing a cytotoxic T lymphocyte (CTL) *in vitro* that is specific for a tumor cell expressing HLA-A2, A1 or A11 comprising contacting a precursor CTL with:

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 under conditions that generate a CTL response to said tumor cells; or

at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354 under conditions that generate a CTL response to said tumor cells.

22. A process for inducing a CTL response *in vitro* that is specific for a tumor cell expressing HLA-A2, A1 or A11, said process comprising contacting a precursor CTL with a cell comprising

a polynucleotide comprising a nucleic acid sequence encoding at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354; or

a polynucleotide comprising a nucleic acid sequence encoding at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354.

23. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing HLA-A2, A1 or A11, said process comprising administering CTLs induced by the methods of claims 21 or 22 in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines.

24. A method for treating a subject with cancer, said cancer characterized by tumor cells expressing any class I MHC molecule and a gene coding for an epitopic sequence of at least one of SEQ ID NO: 355 to 693, whereby the CTLs of claim 20 are administered in an amount sufficient to destroy the tumor cells through direct lysis or to effect the destruction of the tumor cells indirectly through the elaboration of cytokines.

25. The method of claim 1, 13, 19, 20, 23 or 24 wherein said cancer is carcinoma.

26. The method of claim 1, 13, 19, 20, 23 or 24 wherein said cancer is ovarian carcinoma.

27. A method for treating a subject with cancer, said method comprising:

stimulating the production of antibodies for use in passive immunotherapy, wherein said antibodies react with

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354; or

at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354.

28. The method of claim 27, wherein said antibodies are recombinant antibodies.

29. A method for diagnosing the presence of cancer in a subject comprising obtaining a tissue sample from said subject; and

detecting

at least one polypeptide comprising an epitopic peptide comprising an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354; or

at least one polypeptide comprising an epitopic peptide comprising one amino acid difference from an amino acid sequence selected from the group consisting of SEQ ID NO: 1 to 354;

in said sample.

30. The method of claim 29, wherein said polypeptides are detected with an antibody.

31. The method of claim 1 wherein said polypeptide comprises at least two epitopic peptides.

32. The method of claim 31 wherein said polypeptide comprises at least three epitopic peptides.

33. The method of claim 31, said polypeptide comprising a first epitopic peptide and a second epitopic peptide, wherein said first epitopic peptide comprises the amino acid sequence of SEQ ID NO: 1 to 354 and said second epitopic peptide comprises an amino acid sequence selected from the group consisting of SEQ ID NOS: 1 to 354.